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THE SV–40 VIRUS: HAS TAINTED POLIO VACCINE CAUSED AN INCREASE IN CANCER

HEARING

BEFORE THE

SUBCOMMITTEE ON HUMAN RIGHTS AND WELLNESS

OF THE

COMMITTEE ON
GOVERNMENT REFORM

HOUSE OF REPRESENTATIVES

ONE HUNDRED EIGHTH CONGRESS

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THE SV–40 VIRUS: HAS TAINTED POLIO VACCINE CAUSED AN INCREASE IN CANCER

WEDNESDAY, SEPTEMBER 10, 2003

HOUSE OF REPRESENTATIVES,
SUBCOMMITTEE ON HUMAN RIGHTS AND WELLNESS,
COMMITTEE ON GOVERNMENT REFORM,
Washington, DC.

The subcommittee met, pursuant to notice, at 2:30 p.m., in room 2154, Rayburn House Office Building, Hon. Dan Burton (chairman of the subcommittee) presiding.

Present: Representatives Burton, Watson, and Cummings.

Staff present: Mark Walker, staff director; John Rowe and Brian Fauls, professional staff members; Mindi Walker, professional staff member and clerk; Nick Mutton, press secretary; Sarah Despres, Tony Haywood, and Jeff Baran, minority counsels; and Cecelia Morton, minority office manager.

Mr. BURTON. Good afternoon. A quorum being present, the Subcommittee on Human Rights and Wellness will come to order, and I ask unanimous consent that all Members and witnesses' written and opening statements be included in the record, and without objection so ordered. I ask unanimous consent that all articles, exhibits and extraneous or tabular material referred to be included in the record, and without objection so ordered. And we may have some other Members that may want to come. I don't know. We have invited them who are interested in the vaccination issue. If they come I ask unanimous consent that they be allowed to participate and we'll enumerate them as they come assuming they are here.

Immunization to protect people from infectious diseases was one of the greatest public health advances of the 20th century. I don't think anybody argues with the fact that it's made us the luckiest people in the world as far as health is concerned. However, immunization is a very different medical procedure than treating an active disease or injury. Immunizations introduce a potentially disease causing agent into a healthy body and all experts agree that no immunization is without risks.

This is a situation where government policy overrides individual rights. With very few exceptions, immunizations are mandatory. Infants and young children have absolutely no choice in the matter and their parents rarely have a choice. Government mandates require vaccination before admission to day care, school or college. Just last week, here in Washington nearly 10,000 children were turned away on the first day of school because their immunization records were not up to date. They couldn't go to class until they got their shots.

Those in military service get another battery of shots. When freedom of choice conflicts with government edicts, the government nearly always prevails. Because immunizations are mandatory, government agencies at all levels have a duty to exercise the utmost care in the approval, administration and post administration surveillance of vaccines. In fact, the Public Health Act of 1902 imposed a duty upon the Public Health Service to, "ensure the safety, purity and potency of vaccines." "Ensure" is a very strong word. However, doing anything less is a breach of the public trust and could destroy the public's confidence in vaccines.

The development of the polio vaccines in the 1950's and early 1960's was especially welcome because of the devastating toll of death, disability and suffering that polio caused. I can remember my mother wouldn't let me go outside, was worried about flies getting in water that might infect you. And I remember those horrible, horrible machines that children had to live in for the rest of their lives. It was just tragic. So the polio vaccine really was beneficial to mankind as well as U.S. citizens.

However, some parents and a growing number of scientists now believe that the government did not ensure the purity, potency, and safety of some of the polio vaccines and that a breach of the public trust did in fact occur. There is no dispute that millions of Americans received polio vaccines that were contaminated with the virus called Simian Virus 40, or SV–40. There also is no dispute that SV–40 is capable of causing cancer, but there is a major dispute as to how many Americans may have received the contaminated vaccine, with estimates ranging from 4 million to 100 million people. There is also a major dispute as to when the polio vaccine supply got cleaned up. In addition, nobody knows how many people got sick or died because of the contaminated vaccines.

This subcommittee's efforts to give a full and fair hearing to this important issue today are somewhat impaired by the lack of participation by some key Federal health agencies. The Food and Drug Administration informed our staff that they were having trouble locating FDA staff with sufficient knowledge to be of much help and that they needed more time to study it. They promised to submit a statement for the record within the next 2 weeks. Well, we'll anxiously watch for their statement and we will give the appropriate FDA personnel the opportunity to appear before this subcommittee down the road when those things have been located.

The Centers for Disease Control and Prevention indicated that they don't keep records on things that happened 40 or 50 years ago and that they could not be very helpful. That in and of itself raises a serious question in my mind. We're not talking about the common cold here. We are talking about polio, the most devastating epidemic of the first half of the 20th century. We're talking about tainted vaccines that were given to millions of American children and young adults, and I think the FDA and CDC need to look a little harder for their records.

The National Cancer Institute has sent a representative in the person of Dr. James Goedert. Did I pronounce that right?

Dr. GOEDERT. Goedert. The 'o' is silent.

Mr. BURTON. OK. We thank you, Doctor, for your appearing and we thank your agency for sending you to testify today. I also want to thank the other witnesses that are here to testify and I look forward to hearing your testimony. And I understand Dr. Engels is here with you. We appreciate you coming, Doctor, and we will accept testimony and answers of questions from you as well. And I want to thank the other witnesses who are here and look forward to hearing their testimony.

[The prepared statement of Hon. Dan Burton follows:]

Opening Statement
Chairman Dan Burton
Subcommittee on Human Rights and Wellness
Hearing entitled, "The SV40 Virus: Has Tainted Polio Vaccine Caused an Increase in Cancer?"
September 10, 2003

Immunization to protect people from infectious diseases was one of the greatest public health advances of the twentieth century.

However, immunization is a very different medical procedure than treating an active disease or injury. Immunizations introduce a potentially disease-causing agent into a healthy body, and all experts agree that no immunization is without its risks.

This is a situation where government policy overrides individual rights. With very few exceptions, immunizations are mandatory. Infants and young children have absolutely no choice in the matter, and their parents rarely have a choice.

Government mandates require vaccinations before admission to day care, school, or college. Just last week, Nearly 10,000 Washington, DC school kids were turned away on the first day of school because their immunization records were not up-to-date. They couldn't go to class until they got their shots. Those in military service get another battery of shots.

When freedom of choice conflicts with government edicts, government nearly always prevails.

Because immunizations are mandatory, government agencies at all levels have a duty to exercise the utmost care in the approval, administration, and post-administration surveillance of vaccines. In fact, the Public Health Act of 1902 imposed the duty upon the Public Health Service to: "*Insure the safety, purity, and potency*"of vaccines.

Insure is a very strong word. However, doing anything less is a breach of the public trust, and could destroy the public's confidence in vaccines.

The development of polio vaccines in the 1950s and early 1960s was especially welcome, because of the devastating toll of death, disability, and suffering that polio caused. However, some parents, and a growing number of scientists, now believe that the government did not insure the purity, potency, and safety of some polio vaccines, and that a breach of the public trust did in fact occur.

There is no dispute that millions of Americans received polio vaccines that were contaminated with a virus called simian virus 40 (SV40).

There also is no dispute that SV40 is capable of causing cancer.

But there is a major dispute as to how many Americans may have received the contaminated vaccine, with estimates ranging from 4 million to 100 million.

There is also a major dispute as to when the polio vaccine supply got cleaned-up. In addition, nobody knows how many people got sick or died because of the contaminated vaccines.

This Subcommittee's efforts to give a full and fair hearing to this important issue today are somewhat impaired by the lack of participation by some key Federal health agencies.

The Food and Drug Administration informed our staff that they were having trouble locating FDA staff with sufficient knowledge to be of much help, and that they needed more time to study it. They promised to submit a statement for the record within the next two weeks. We'll anxiously watch for their statement, and we'll give the appropriate FDA personnel the opportunity to appear before this Subcommittee when they have been located.

The Centers for Disease Control and Prevention indicated that they don't keep records on things that happened 40 or 50 years ago, and that they could not be very helpful. That in and of itself raises a serious question in my mind. We are not talking about the common cold here. We are talking about polio – the most devastating epidemic of the first half of the twentieth century – we are talking about tainted vaccines that were given to millions of American children and young adults! I think the FDA and the CDC need to look a little harder for their records.

The National Cancer Institute has sent a representative in the person of Dr. James Goedert. We thank you Dr. Goedert for appearing, and we thank your agency for sending you to testify today.

I also want to thank the other witnesses who are here and I look forward to hearing your testimony.

Mr. BURTON. Would you gentlemen please raise your right hands and stand?

[Witnesses sworn.]

Mr. BURTON. Doctor, we'll start with you. Dr. Goedert. Go ahead, Doctor. And we would like to keep our testimony as much as possible to 5 minutes because we want to get on with questions and we may have more votes.

STATEMENT OF DR. JAMES GOEDERT, CHIEF OF VIRAL EPIDEMIOLOGY, NATIONAL CANCER INSTITUTE, ACCOMPANIED BY DR. ERIC A. ENGELS

Dr. GOEDERT. Mr. Chairman, I appreciate the opportunity to appear before you. My name is James Goedert. I'm a physician, a graduate of Loyola University Medical Center, in Maywood, IL, with training and board certification in internal medicine and medical oncology. Like everyone here, I have seen suffering and death from cancer, including close family members. To reduce suffering and death from cancer I have dedicated my professional career, over 23 years with the National Cancer Institute at the National Institutes of Health, conducting research on the causes and prevention of cancer.

Today we consider two related but scientifically distinct questions: Is cancer associated with the inadvertent contamination of the early polio vaccines with SV–40, and do people with cancer have evidence of SV–40 infection irrespective of the source? We have and continue to take both questions seriously. Our current Division Director, Dr. Joseph Fraumeni, immediately recognized the potential impact of polio virus contamination with SV–40. In 1963 he studied and found no difference in cancer risk associated with the use of the contaminated vaccine. As you know, cancer, can take years to develop so this study could not be the final word.

During the ensuing 40 years, we and many others have continued to study populations exposed to SV–40 contaminated vaccines, including children, the offspring of women vaccinated during pregnancy, military servicemen and the population of Denmark. Though some of these studies are ongoing, one point is clear. They have consistently found that recipients of SV–40 contaminated vaccines do not have an increased risk of cancer.

Turning to the second question of SV–40 in people irrespective of the source, the reported detection of SV–40 DNA in two types of brain cancer in children and in mesothelioma and osteosarcoma tissue prompted us to initiate laboratory studies. In 48 mesotheliomas from the archives of the Armed Forces Institute of Pathology we found no SV–40 DNA despite the use of two laboratory methods, each able to detect 10 or fewer molecules of SV–40 DNA. Other highly experienced laboratories also did not detect SV–40 DNA in mesothelioma. Still others were detecting SV–40 DNA in a wide variety of tumors and at the same time at extraordinarily high rates in normal blood and tissue samples.

It should be noted that our studies and those of others use the PCR technique, a very powerful method for detecting minute amounts of DNA, but one also prone to false positive results if handling procedures and negative controls are lacking.

To clarify the disparate results we and our colleagues at the Food and Drug Administration organized an international SV–40 working group, including laboratories that had previously detected SV–40, some that had not and some that were new to the field. Fundamental to the international working group study was the development of the study protocol that is included in our written materials. This protocol is the end product of extensive in-depth face-to-face discussions and correspondence. All of the participating laboratories and other collaborating units contributed to the development of its specification.

Three results from the international working group study are of note. First, the PCR assays were highly sensitive and specific in SV–40 positive and negative control specimens respectively. Second, SV–40 DNA was detected reproducibly in zero of 25 fresh frozen, optimally handled mesothelioma tissues. Third, despite what were thought to be adequate safeguards SV–40 DNA contaminated a batch of normal cells in one laboratory and SV–40 DNA contaminated the PCR reagents in a second laboratory. These events illustrate the ease with which a few DNA SV–40 DNA molecules can creep into an experiment and be detected by PCR.

The bottom line of the international working group study is that the SV–40 PCR tests worked well but there was no reproducible detection of SV–40 DNA in mesothelioma. We also evaluated the possibility that SV–40 is circulating in people without cancer. In 166 urine samples from men in Washington, DC, or New York City we compared the prevalence of the two human polyoma viruses, called BK virus and JC virus, to the prevalence of SV–40. We found that 14 percent of these men were excreting BK virus, 34 percent were excreting JC virus and not one was excreting SV–40. Even in people with advanced HIV/AIDS we found no excretion of SV–40. This work and other studies would indicate that SV–40 does not circulate in the general population today.

Our results should be considered in the context of the report of the Immunization Safety Review Committee of the National Academy of Sciences Institute of Medicine [IOM], as included in our written materials. This is as prestigious a body of scientists as can be assembled. Our approaches and findings are wholly consistent with the IOM's conclusions and recommendations which we endorse. IOM concluded, "that the evidence is inadequate to accept or reject a causal relationship between SV–40 containing polio vaccines and cancer." The IOM had five research recommendations that are provided in our written materials and that I will gladly discuss.

To conclude, we remain committed to identifying the causes of cancer. If SV–40 was found to cause human cancer tests could be developed, people could be screened and perhaps even treatments could be improved. However, our work and that of excellent research centers in the United States and Europe currently reveals no association between SV–40 and cancer in people. We do not consider the matter settled, as new technologies could afford new insights. Irrespective of new technology, future studies must adhere strictly to tightly reasoned, stringently defined research protocols. We invite others to replicate the international working group study, including successful masking and sufficient numbers and types of positive and negative controls.

In sum, on the basis of the available data we do not have evidence that SV–40 causes human cancer. Only through rigorous, disciplined and transparent science will we find the insight and the means to prevent and relieve the suffering of the cancers being considered by the committee today.

That concludes my statement. I'll be pleased to answer any questions.

[The prepared statement of Dr. Goedert follows:]

Testimony
Before the Subcommittee on Wellness and Human Rights
Committee on Government Reform
United States House of Representatives

Research on SV 40 Exposure and the Development of Cancer

Statement of

James Goedert, M.D.

Chief, Viral Epidemiology Branch
Division of Cancer Epidemiology and Genetics
National Cancer Institute
National Institutes of Health
U.S. Department of Health and Human Services

For Release on Delivery
Expected at 2:00 PM on
Wednesday, September 10, 2003

Introduction ~~Paragraph~~

Thank you, Mr. Chairman, for inviting the National Cancer Institute (NCI), an agency of the National Institutes of Health, Department of Health and Human Services, to testify before the Subcommittee today. I am James Goedert, M.D., Captain, US Public Health Service, and Chief of NCI's Viral Epidemiology Branch (VEB) in the Division of the Cancer Epidemiology and Genetics.

Our Branch conducts population-based epidemiology research to clarify the relationship of infectious agents, especially viruses, to human cancer and other conditions. Viruses may cause or increase the risk of cancer through several mechanisms. These include allowing uncontrolled cell division, blocking DNA repair, and altering the immune system. While some viruses have been known to be related to cancer for many years, new infectious carcinogenic agents continue to be found. The Branch utilizes the principles of both infectious and chronic disease epidemiology, supported by collaborative statistical modeling and laboratory investigations.

Some cancer-associated viruses appear to rarely cause cancer among exposed persons. Other viruses substantially increase the risk for cancer and the burden of this disease in the population. Knowing which cancers are associated with the different viruses can help promote targeted cancer screening, early detection, and treatment. If cancer is more common in people with exposure to the virus, then that suggests that the virus might cause the cancer. Similarly, if people with cancer are more likely to have been infected with the virus than healthy people, this also suggests that the virus could cause cancer.

Like all scientific research, individual epidemiologic studies cannot provide a definitive answer about the relationship between an exposure and the development of cancer. Rather, epidemiologic and other scientific research studies build a body of evidence -- supported over the years by larger and more rigorous research studies – that ultimately convince the scientific community, and the policy-makers of state and Federal governments, of the existence of such a relationship. At this time, our opinion is that the body of evidence is inconclusive as to the role of SV40 in the development of cancer.

SV40 in Early Vaccines

Simian virus 40 (SV40) is a virus that infects several species of monkeys and typically does not cause disease in them. The virus was discovered in 1960 in rhesus macaque monkey kidney cells that were used in the production of the original Salk and Sabin polio vaccines (1). Since the mass immunization program for polio began in 1955, before the discovery of the virus, contaminated vaccine lots were inadvertently used for the first few years of the program.

When reports appeared in 1961 that injecting SV40 into hamsters could cause tumors (2-5), the United States (U.S.) government instituted a screening program requiring that all new lots of poliovirus vaccine be free of SV40 because of concerns about possible adverse effects on human health. Already-produced vaccine may have been used through 1962, but U.S. polio vaccine has been free of SV40 since 1963 (6). Published results of testing confirm that no SV40 has been found in U.S. polio vaccine lots tested after 1972 (47). The polio vaccine currently used in the U.S. is produced under carefully regulated conditions designed and enforced by the Food and Drug Administration to ensure that contamination with SV40 does not occur. As a result of the earlier contamination, however, it is estimated that more than 10 million to 30 million people vaccinated in the United States from 1955 through early 1963 were inadvertently exposed to live SV40 (6).

SV40 in Animals

In laboratory animal studies, the SV40 virus has been found to cause malignancies (mesothelioma, ependymoma, osteosarcoma, and leukemia and lymphoma) in newborn rodents, particularly hamsters, exposed to high levels of the virus (50). However, the question of whether SV40 causes human cancer is unsettled, as published data are contradictory.

Follow-up of Vaccine Recipients Shows No Excess of Cancer

Over the last four decades, an intense research effort has been made to determine whether the exposure to SV40 through polio vaccination has caused cancer in people. Up through the early 1990's, epidemiologic studies involving decades of observations and millions of people in the U.S. and Europe have failed to detect an increased cancer risk in those likely to have been exposed to the virus. These include a long-term Swedish study, which followed 700,000 people who received SV40-contaminated vaccine (7), a German study with 22 years of follow-up of 886,000 persons who received the contaminated vaccine as infants (8), a 20-year study of 1,000 people in the United States inoculated during the first days of life with contaminated vaccines (9), a 30-year follow-up of approximately 10 percent of the entire U.S. population (using data from the National Cancer Institute's Surveillance, Epidemiology, and End Results registry) (10), and a 40-year follow-up study of 1.8 million recipients of a widely contaminated Danish polio vaccine (38) .

SV40 is Variably Detected in Human Tumors

The issue of SV40 and cancer has surfaced in the last few years when some laboratories, using an extremely sensitive molecular biology technique, the polymerase chain reaction (PCR), found traces of SV40 DNA in some rare human tumors including pleural mesothelioma (a cancer of the lining of the lung), osteosarcoma (a type of bone cancer), ependymoma and choroid plexus tumors of the brain, and recently non-Hodgkin lymphoma (12-29). Other studies reported that SV40 T-antigen, a viral protein, binds to human tumor suppressor proteins such as p53 and RB (30-32), suggesting a possible carcinogenic mechanism. Not all studies, however, have found that SV40 can be detected in human cancer (33-37, 39-46). When detected, SV40 has been found at very low levels (40), raising questions about the biological role that SV40 could play and suggesting that reported detection could be a laboratory artifact. Finally, some studies have found SV40 in a wide range of other tumors and normal tissues (16), which raises further questions about the biological interpretation of positive findings.

Laboratory Studies Result in Controversy and Uncertainty

In order to resolve why some laboratories detect traces of SV40 in mesothelioma while others do not, an International SV40 Working Group, which included the majority of laboratories studying SV40 in human tissues, was formed in 1997. Nine laboratories from the working group agreed to participate in a study, funded and organized by NCI. Under a tightly reasoned, thoroughly vetted, and tightly enforced research protocol (Appendix 1), each group was given 25 paired-duplicate samples of human mesotheliomas, a single set of 25 normal lung tissue samples, and positive and negative control samples. All the samples were masked (prepared and labeled so that the human tumors and controls could not be distinguished). Each laboratory used one or more assays for detecting SV40, many of which had been used to detect SV40 previously. The results (36) showed that none of the mesothelioma specimens was consistently positive for SV40 across all laboratories. New methods that can be used widely and easily to reliably detect the presence of SV40 DNA in human tissues are needed.

Recent and Ongoing Research

Since the early 1990's, the NCI and other investigators continue to evaluate the possible link between SV40 infection and human cancers. Our Branch is monitoring populations known to have been exposed to SV40-contaminated vaccines, and some of our recently completed and ongoing studies are described below. Additional extramural grant-supported studies funded by NCI to evaluate the possible relationship of SV40 to cancer are underway (http://researchportfolio.cancer.gov/). Other institutes at the National Institutes of Health are also funding a wide range of studies related to SV40 (https://www-commons.cit.nih.gov/crisp).

- *Mesothelioma in the U.S. (37)* Using data from the Surveillance, Epidemiology and End Results (SEER) population, which is a 10% sample of the entire U.S. population, NCI examined the incidence of mesothelioma of the lining of the lung (the pleura), with a particular focus on individuals' ages during the 1955-1963 interval when poliovirus vaccines were contaminated with SV40. The rate of pleural mesothelioma was highest among men over age 75, who were least likely to have received SV40-contaminated vaccine and most likely to have been exposed to asbestos, a known cause of mesothelioma. In middle age individuals, between 25 and 54 years of age, who were infants or children during 1955-1963 and most likely to have received SV40-contaminated poliovirus vaccine, mesothelioma rates have been low and even decreasing. Females, although equally exposed to SV40-contaminated vaccines during childhood, had much lower mesothelioma rates, probably because they have been much less exposed to asbestos. We concluded that after almost 40 years of follow-up, U.S. cancer incidence data have not shown an increased incidence of pleural mesothelioma among the age groups that were exposed to SV40-contaminated poliovirus vaccine.

- *Cancer incidence in Denmark. (38)* In Denmark, inactivated poliovirus vaccine was first administered in April 1955, a few weeks after vaccination campaigns began in the U.S. Because of the urgency of the epidemic, a concerted effort was mounted to administer poliovirus vaccine to a large proportion of the population, and Denmark maintained a high level of vaccination through the early 1960s. NCI and Danish investigators recently examined cancer incidence in Denmark as a function of birth year and calendar year, which served to identify exposure to early poliovirus vaccine. Importantly, review of 1960s Danish records identified widespread SV40-contamination of previously utilized Danish poliovirus vaccine, which, unlike in the U.S, was grown in pooled kidney tissue from dozens of monkeys. A further strength of this study was the high quality of Denmark's nation-wide data on cancer incidence, which go back to 1943. Overall cancer incidence was actually lower in SV40-exposed individuals (age-adjusted relative risks 0.86, 95%CI 0.81-0.91, and 0.79, 95%CI 0.75-0.84, for those exposed as infants or children, respectively, compared with those unexposed). No increased risk was seen for specific outcomes, including mesothelioma, bone tumors, brain tumors, and non-Hodgkin's lymphoma (NHL).

- *Brain tumors in northern India.(39)* A study involving a population uniquely exposed to SV40 - people living in northern India - was published recently. It is unclear whether humans can be infected with SV40 but, if this occurs, human infection might be especially common in northern India, where contacts between humans and SV40-infected monkeys frequently occur. NCI researchers and collaborators tested for the presence of SV40 in 47 archived samples of choroid plexus tumors and ependymomas, which are rare human brain tumors reportedly linked with SV40 (15, 17), from the All India Institute of Medical Sciences in northern India. Non-malignant brain tissues were included as negative controls, and laboratory workers were masked to the case-control status of specimens. A further strength of the study was the use of real-time polymerase chain reaction to quantify SV40 and cellular DNA detected in specimens. Investigators did not find SV40 in any of the tumors. Given the PCR assay's sensitivity, SV40 would have been detected if it was present in at least one copy per 10 cells.

- *Case-control study of non-Hodgkin's lymphoma in Spain. (45)* In a case/control study in Spain involving 520 lymphoma cases and 587 controls, researchers tested blood samples from cases and controls for the presence of antibodies to SV40. If SV40 circulates in human populations and is implicated in lymphomas, SV40 serum antibodies might be detected at high levels in lymphoma cases. However, the researchers found no increased antibody levels to SV40 detected in lymphoma cases vs. the controls. Overall, SV40 antibody levels were low in both cases and controls. Additional testing suggested that a large part of these antibodies may be antibodies to the human virus BK, and not to SV40. Because the DNA of the SV40 virus is nearly 70 percent identical to the BK virus, it is difficult to distinguish between antibodies to the two viruses. Most humans carry antibodies to BK in their blood, since the virus commonly infects humans as children. BK, however, is not associated with any disease in healthy people

- *Polio vaccination history in brain tumor patients. (46)* In another case/control study involving 782 brain tumor cases and 799 controls (46), the risk of developing glioma, meningioma, or acoustic neuroma was not associated with having reported receiving either injected or oral poliovirus vaccine during the time period (1955-1963) when vaccines were contaminated with SV40.

- *Follow-up of recipients of U.S. Army's adenovirus vaccine (48)* To eliminate severe outbreaks of respiratory illness in basic training camps, the U.S. Army administered an inactivated adenovirus vaccine, grown in monkey kidney tissue, to entering service personnel in 1960-61. Evidence is compelling that this vaccine was widely contaminated with live SV40. Adenovirus grows extremely poorly in monkey kidney tissue without the presence of SV40 as a "helper virus." This situation is unlike poliovirus vaccine contamination, which in the U.S. did not occur uniformly, because SV40 was not a necessary cofactor for poliovirus replication *in vitro*. As with poliovirus vaccine, formalin-inactivation did not completely inactivate contaminating SV40.

  NCI investigators are conducting a retrospective cohort study of Army servicemen from this era. Cases of mesothelioma, brain tumors, and non-Hodgkin's lymphoma in military veterans will be linked to military service records to determine which individuals entered Army service on a date that corresponded to the Army's use of this vaccine. An additional advantage of the study design is the attained age of the men who entered the Army in 1959-61; by the 1990s, they would have reached an age when mesothelioma incidence becomes appreciable. Results from this study should be available soon.

- *Case-control study of childhood cancer.* NCI investigators are conducting a case-control study of childhood cancer that should be informative with respect to the role of SV40 in human cancer. In the U.S. during the 1950s and 1960s, pregnant women were frequently given inactivated poliovirus vaccine, potentially leading to infection of their children with SV40 *in utero* or soon after birth. Given the carcinogenic potential of SV40 in newborn laboratory animals, follow-up of children whose mothers were vaccinated during pregnancy represents a unique means of determining whether SV40 causes human cancer.

  To pursue this line of inquiry, NCI has organized a study of SV40 and childhood cancer nested in the Collaborative Perinatal Project (CPP) cohort study. CPP enrolled pregnant women and their subsequently-born children in 1959-66 at 12 U.S. university medical centers. The cohort comprises 54,796 children born to 44,621 mothers. Enrolled mothers had study visits scheduled as part of their prenatal care, and detailed data on vaccinations during pregnancy reveal that 22.5% of CPP children were exposed *in utero* to pre-1963 poliovirus vaccine, 17.0% were exposed *in utero* to 1963+ poliovirus vaccine, and 60.5% of children were unexposed.

  Through age 8 years, 52 CPP children developed cancer (18 neural tumors, 22 hematologic malignancies, 12 miscellaneous tumors). In a nested case-control study, paired sera (from early and late in pregnancy) have been selected from the 50 mothers of these children with available specimens and from 200 CPP control mothers. These sera are being evaluated for SV40 antibodies using an SV40 plaque neutralization assay and a virus-like particle-based (VLP) enzyme immunoassay.

- *Case-control study of non-Hodgkin's lymphoma in the U.S.* The possibility that SV40 causes a substantial fraction of non-Hodgkin's lymphoma in the U.S. was recently raised by two studies reporting the molecular detection of SV40 DNA in 40-50% of tissues. However, confirmatory evidence of SV40 infection (e.g., SV40 antibody) in non-Hodgkin's lymphoma cases was lacking, and SV40 was detected in tissues other than non-Hodgkin's lymphoma in one of the studies. In addition, these studies could not provide an estimate of the relative risk associated with SV40 infection.

NCI and laboratory collaborators at two institutions are pursuing this question further using samples collected in a case-control study of non-Hodgkin's lymphoma in the U.S. This study includes approximately 800 HIV-uninfected non-Hodgkin's lymphoma cases and 700 age-matched population controls from the NCI-Surveillance Epidemiology and End Results (SEER) Case-Control Study of non-Hodgkin's lymphoma SV40 and BK serostatus will be assessed using VLP assays. Strengths of the U.S.-based study include the widespread exposures of the U.S. population to SV40-contaminated poliovirus vaccine and the representative nature of the non-Hodgkin's lymphoma cases (sampled consecutively at four SEER registry sites) and population-based controls. This study will help answer whether SV40 infection is more common in persons with non-Hodgkin's lymphoma than controls and thus provide evidence on whether SV40 might cause non-Hodgkin's lymphoma. Importantly, finding low SV40 seroprevalence in cases (i.e., substantially less than 40%, reported previously) would argue against SV40 as a cause of non-Hodgkin's lymphoma.

- *AIDS-associated non-Hodgkin's lymphoma. (49)* Recent reports of the detection of SV40 DNA sequences in 40% of AIDS-associated non-Hodgkin's lymphomas prompted NCI investigators to examine whether, among individuals with AIDS, those exposed to SV40-contaminated poliovirus as children had an increased risk for non-Hodgkin's lymphoma. Non-Hodgkin's lymphoma incidence was estimated for two cohorts with AIDS: persons born in 1958-61 (exposed to SV40-contaminated poliovirus vaccine as infants) or born in 1964-67 (born after the vaccine was cleared of SV40 and thus unexposed). Non-Hodgkin's lymphoma incidence was marginally higher in the exposed cohort (unadjusted relative risk 1.15, 95%CI 0.99-1.34, vs. unexposed cohort). Notably, however, the exposed cohort developed AIDS slightly earlier than the unexposed cohort (mean year of onset 1992 vs. 1993). Due to the temporal evolution of the U.S. AIDS epidemic, the two cohorts thus differed in composition, with the exposed cohort having more males and whites, who are known to be at increased risk of non-Hodgkin's lymphoma irrespective of AIDS or vaccination status, than the unexposed cohort. Also, exposed individuals were, on average, five years older at AIDS onset than unexposed individuals. With adjustment for these differences, non-Hodgkin's lymphoma incidence was identical in exposed and unexposed individuals (relative risk 0.97, 95%CI 0.79-1.20).

- *SV40 infection in primate workers.* Evaluation of persons with occupational exposure to SV40, i.e., exposure to macaques, would be valuable in documenting whether SV40 infection can occur in humans. Rhesus macaques are universally infected with SV40 by adulthood, and cynomolgus macaques are readily infected through rhesus contacts in captivity. Humans working with monkeys could become infected with SV40 via bites, scratches, or exposure to contaminated urine.

With collaborators at the Centers for Disease Control and Prevention (CDC) and Johns Hopkins, NCI is undertaking a pilot study to examine whether workers in primate centers and zoos in North America display serologic evidence for SV40 infection. Investigators will determine whether SV40 seroprevalence is higher in workers exposed to primates than controls. Additionally, SV40-seropositive subjects will be further characterized, with regards to the specificity of SV40 antibody reactivity (i.e., evaluation of BK virus reactivity) and SV40 antibody titer. Evidence for SV40 infections will prompt additional studies that would include more detailed exposure and health outcome data and other types of biological specimens.

IOM Report

The Institute of Medicine (IOM) of the National Academy of Sciences issued a report in October 2002 (50), which concluded that scientific "evidence is inadequate to accept or reject a causal relationship between SV40-containing polio vaccines and cancer." (p. 11, Executive Summary). The committee stated that the "biological evidence is of moderate strength that SV40 exposure could lead to cancer in humans under natural conditions" and that "biological evidence is of moderate strength that SV40 exposure from the polio vaccine is related to SV40 infection in humans." (p. 11, Executive Summary)

Based on these conclusions, the Institute of Medicine made the following research recommendations: (Appendix II)

> *Research*
> The committee recommends development of sensitive and specific serologic tests for SV40.
>
> The committee recommends the development and use of sensitive and specific standardized techniques for SV40 detection.
>
> The committee recommends that once there is agreement in the scientific community as to the best detection methods and protocols, pre-1955 samples of human tissues should be assayed for presence or absence of SV40 in rigorous, multi-center studies.
>
> The committee recommends further study of the transmissibility of SV40 in humans.
>
> Until some of the technical issues are resolved, the committee does not recommend additional epidemiological studies of people potentially exposed to the contaminated polio vaccine.

Our Branch will continue to collaborate with others in multidisciplinary research fields to settle the uncertainties that remain, and to pursue new leads to clarify the relationship between SV40 and human cancer, if any.

Closing

As we move forward to resolve the uncertainties in this field, researchers will need to understand what the detection -- or lack of detection-- of SV40 DNA in tumors implies. In recognition of the IOM's recommendation that molecular methods for SV40 detection be standardized, future studies will need to include sufficient numbers and types of positive- and negative-control specimens and to make the status of the specimens (i.e., controls, tumors, and others) unknown to the persons performing the laboratory analyses. Valuable data may come from newly available serologic techniques, but only with rigorous study designs that mask case-control status and include sufficient number of subjects. To study whether SV40 is in the human population and, if so, its modes of transmission, epidemiologic studies could be conducted if assays, such as the new SV40 antibody techniques, are shown to be highly sensitive, specific, and reproducible.

Because of the widespread exposure to SV40 through contaminated vaccines, the question of whether SV40 causes some human cancers has substantial public health implications. However, the types of claimed to be linked to SV40 have been and continue to be very rare. In addition, SV40 prevalence in the general population is unknown, and detection of SV40 in humans is controversial. We remain committed to helping resolve these questions.

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Mr. BURTON. Thank you, Dr. Goedert. And, Doctor, can we just rely on you for the answering of questions unless you have something——

Dr. ENGELS. Yes.

Mr. BURTON. OK. Thank you. When did government health agencies first discover that SV–40 was in the polio vaccine supply?

Dr. GOEDERT. The virus itself was discovered in 1960. And in 1961, Dr. Eddy detected an occurrence of cancer in rodents that were injected with the vaccine preparation.

Mr. BURTON. Hamsters I think, wasn't it?

Dr. GOEDERT. Newborn hamsters.

Mr. BURTON. You're saying that there's no proof that the SV–40 that was in those vaccines has caused cancer? Is that what you're saying?

Dr. GOEDERT. I'm saying that the issue remains open because essentially the criteria for causality certainly have not been fulfilled in terms of reproducibility, specificity and many other criteria, so I think it remains an open question.

Mr. BURTON. You know we've had some real problems with the FDA and other agencies in the past because there's been concern that there's too much influence exerted by pharmaceutical companies on our health agencies, and I'm not saying that's the case with you, Doctor, but it's real troubling because 60 laboratories I understand around the world have done testing and said that the SV–40 is a cause of cancer, and I can't understand how 60 laboratories could be wrong and the FDA be correct.

Can you explain it to me? And these are not fly by nights. These are some leading scientists. Let me give you just a few names, OK? Dr. Carbone. He said that they found SV–40 in a large portion of tumors. Some of the percentages were 60, 63 and 41 percents in three tests. Dr. Cristaudo. He found cancer in—or SV–40 in 72 and 52 percent of the cancers in two tests. Dr. DeLuca. He found 86 percent. Dr. Mayall found 45 percent. However, Dr. Strickler, I guess he worked for you at one time and is now a consultant or does consulting with the FDA, or does he have some relationship with the FDA now?

Dr. GOEDERT. I'm unfamiliar with him.

Mr. BURTON. Who's he with now? Albert Einstein Institute. Does he do any contracting or anything with you?

Dr. GOEDERT. Dr. Strickler is a former postdoctoral fellow from my branch who is a—I believe an assistant professor at Albert Einstein College of Medicine in New York, and we have until recently collaborated with him on a number of projects subsequent to his departure from our group.

Mr. BURTON. Yes, sir. Well, Dr. Strickler evidently has done some research on this, and he showed that in 1996 there was no evidence that the SV–40 was in any tumors and was the cause of these cancers. And in 2001 he said the same thing. Now, how do you account for the fact that your scientist, he was working for you I think at the time, couldn't find any trace of SV–40 tumors when eminent scientists that I just mentioned to you and many others from 60 different laboratories around the world found many cases of its existence? Why is there that inconsistency? Scientists look at, you know, a lot of the same data. I mean your scientist says no and

60 other laboratories say yes and eminent doctors from those laboratories that have done extensive research say yes. Why that inconsistency?

Dr. GOEDERT. Mr. Chairman, I think reasonable people can disagree, and I'm sure that each individual believes in his or her own data. But the data are contradictory and the field is unsettled. There are 11 studies like ours that find no SV–40. There are four serological; that is, antibody, studies that find no difference between people with cancer and people without cancer. The studies that have found SV–40, not all of them, but many lack the controls and the maskings of specimens that we require to have confidence in our results. Some of the others are internally inconsistent and contradictory and they typically have not been replicated. I mentioned during my opening statement that our own study with nine laboratories had two instances, two events where SV–40 contamination occurred. I think this is the most likely but not the sole explanation for why SV–40 may be detected but not actually related to the tumor.

Mr. BURTON. You know, sometimes our health agencies and even the executive branch and other areas come before our committee and they have what I call selective memory loss or they use terminology that equivocates on an issue, and I know you don't want to do that doctor, but I do want to read something to you.

The institute, the NCI, reassured the medical community over the years that there was no evidence of cancer caused by contaminated polio vaccine. However, in referring to study after study, the Institute of Medicine report of October 2002, just last year, said, "weaknesses in the study limit its contribution to the causality argument."

Were the National Cancer Institute's reassurances over the years that polio vaccine did not cause cancer linked to these now discredited studies?

Dr. GOEDERT. Mr. Chairman, the Institute of Medicine concluded that the data are inconclusive with respect to causality, taking into account all of the available information. Epidemiologic studies are not perfect and it would be more challenging if we had found an association with cancer in a study that was less than ideal.

Mr. BURTON. Well, the IOM says that there were weaknesses in the study and it limited its contribution to the causality argument. Now that you know that study has been partial—and those studies have been partially discredited, what is our health agency going to do to try to fix the problem and is there any new research methods that you guys are proposing over there that wouldn't be inherently flawed?

Dr. GOEDERT. Mr. Chairman, no single study is going to be perfect. We endorse and are following through with the recommendations of the IOM panel. The first recommendation, and we agree it's the most important, is the development of the serological tests that can more clearly define who is likely to be infected and uninfected with this virus. We are working with two university groups on this effort and are following through with the application of a number of different studies.

That said, we have always maintained that the question remains open and we do not say that there is no chance whatsoever that this virus is associated with cancer. We have said that there is no evidence of an excess risk of cancer related to exposure to the contaminated vaccines.

Mr. BURTON. Let me ask just a couple more questions and then I'll yield to you and I'll give you as much time as you like. Eminent doctors, three tests by Dr. Carbone, 60, 63 percent and 41 percent of the cancers they looked at had the SV–40; 72 and 50 percent by Dr. Cristaudo; 86 percent by Dr. DeLuca; 45 percent by Dr. Mayall. The IOM report of October 2002 indicates that the biological evidence is strong, strong, that SV–40 is a transforming virus capable of causing cancer. Does the NCI agree with that?

Dr. GOEDERT. Yes, Mr. Chairman, we do. In animals and in test experiments.

Mr. BURTON. In animals?

Dr. GOEDERT. In hamsters.

Mr. BURTON. In hamsters.

Dr. GOEDERT. Sure.

Mr. BURTON. Does the NCI consider a human being an animal?

Dr. GOEDERT. Mr. Chairman, I'm sorry. Maybe I misunderstood your question.

Mr. BURTON. Well, you said in animals they found that there was a causality.

Dr. GOEDERT. I believe the conclusion——

Mr. BURTON. In hamsters you said.

Dr. GOEDERT. I'm sorry, Mr. Chairman. I'm losing it.

Mr. BURTON. No, you said in hamsters that the evidence was strong that the SV–40 was a cause of tumors, and you said in animals. You said the tests in animals. You were being generic instead of saying hamsters. You said tests in animals.

Dr. GOEDERT. Well, I believe that it's not 100 percent limited to hamsters. I believe there was some lesser evidence in other rodents.

Mr. BURTON. Other rodents. OK. But you're talking about animals. Or rodents.

Dr. GOEDERT. Yes, sir.

Mr. BURTON. Are human beings animals? Are we considered animals biologically?

Dr. GOEDERT. Mr. Chairman, I think that perhaps gets into a little bit of a philosophical question.

Mr. BURTON. Well, I'm talking about from a biological standpoint. The point I'm trying to make is this. If it causes tumors and cancers in rodents and hamsters, if you have other eminent scientists around the world saying that it causes tumors and cancers in human beings, if 60 well known laboratories around the world say that they have scientific evidence that caused cancers and the only one that we know that is saying that no, it didn't, there's no evidence of it, is Dr. Strickler, who used to work for you, that would lead one to believe that there's something wrong. Either the same tests aren't being utilized by our health agencies, or else they're not looking at it fairly.

Dr. GOEDERT. Mr. Chairman, there are 11 studies that have found no SV–40 in those tumors. There are four studies that found no difference in antibody between people with cancer and people without cancer, and the nine laboratories in our studies, none of those were government laboratories, and several of those had previously detected SV–40 and were unable to do so when they met our stringent criteria with respect to the blinding of the specimens and the reproducibility.

Mr. BURTON. Well, let me yield to Ms. Watson and I'll get back to you in a minute.

Ms. WATSON. Thank you so much, Mr. Chairman. Mr. Chairman, immunizations against infectious disease is undoubtedly one of the greatest achievements of our public health. As a result of universal immunization, many diseases that just decades ago threatened sickness, disability and death to large segments of the world's population are no longer serious threats to the public health. Polio is among the greatest examples. Polio primarily affects children under the age of 3 and results in the paralysis of the limbs and/or the respiratory system.

Today, because of immunizations, we are on the verge of global polio eradication. Just seven nations remain polio endemic, with 99 percent of the cases occurring in India, Nigeria and Pakistan. Only funding shortfalls for the World Health Organization's polio eradication initiation stand in the way of global eradication.

Because of the importance of immunization, it is critical that the safety of our vaccine supply be protected against contamination, whether deliberate or inadvertent. With respect to SV–40 contamination of polio vaccines, the Federal health agencies maintain that SV–40 has not appeared in either intravenous or polio vaccine after 1963. Because the vaccine in current use is free of SV–40, the Institute of Medicine in a report released last fall stated that it does not recommend a policy review of polio vaccine on the basis of concerns about cancer risk for exposure to SV–40. Our hearts go out to the victims of cancer and their families who have reasons to believe that SV–40 may have contributed to cancer in their cases, and it is important that we learn as much as we can about the risk of SV–40, sources of human exposure to SV–40, and all biological factors that contributed to development of cancer in humans.

What should not get lost in this discussion today is how vitally important it is that all children and adults receive the vaccinations they need to protect them from the serious health consequences of infectious disease. According to the Centers for Disease Control and Prevention, just 65.5 percent of U.S. children ages 19 to 35 months of age receive all of the vaccinations they should. Numerous States lag well behind the national average.

Maintaining the public's trust in the safety and effectiveness of vaccine is a necessary and important objective that requires vigilance by our Federal health agencies. It is unfortunate that we will not hear from the FDA and the CDC and the Institute of Medicine today. Nevertheless, I hope that today's hearing will play a constructive role in the effort to ensure that the public health benefits of immunization can fully be realized and that vaccines are as safe and effective as they can be.

I must apologize for missing the first part of the testimony. But I am concerned about the discussion I've been part of. And that is we have a section of the scientific community saying that SV–40 can contribute to the onset of cancer and we have a segment of the scientific community saying there's no data that concludes that.

What I would like to know, Doctor, what steps do you see needed to be taken to implement a research agenda that could prove one way or the other? I think we need to take it out of the realm of guessing and continuing to use it if there is speculation that it is cancer, contributing to the onset of cancer. And the tests that have been taken and that you have noted, were these tests adequate in your opinion? And can they ensure all the public that polio vaccine is free of SV–40?

So can you address what is needed down the pike and how we can ensure the public?

Dr. GOEDERT. Madam Congresswoman, as I said in my opening remarks, there's two related but scientifically distinct questions. One has to do with the risk of cancer in people who received contaminated polio virus vaccine, and the other is the association of cancer in people with SV–40 with cancer irrespective of how they may have gotten it.

You're posing a third question which has to do with the safety of the current polio virus vaccines. The FDA would be the people most qualified to answer that. I can tell you information that I have from my preparations here is that since 1963 every lot of vaccine has been tested and certified as free of SV–40 and containing no viable SV–40. In addition, using PCR technology, the FDA itself found no SV–40 DNA molecules in lots that were released between 1972 and 1996. Comparable data have been developed by the FDA equivalent in the United Kingdom and in fact even by Dr. Carbone himself, who the chairman mentioned earlier was unable to detect SV–40 DNA in the current lots, at least current as of when they did them, probably the late 1990's, were unable to detect any trace of SV–40 DNA in those vaccine lots.

With respect to the research agenda, would you like me to address that?

Ms. WATSON. Yes, I would, because I'm hearing conflicting information. The Chair read off a group of scientists who came to a different conclusion than the one that you just reiterated. I possibly would like to see a collaborative effort. And so do you have any suggestions as to how we could get on a research agenda where we could combine findings and come to some final conclusion?

Dr. GOEDERT. Madam Congresswoman, our nine laboratory study which we initiated with the FDA and brought together all of the scientists who had an interest in this field in January 1997 was the—resulted as this SV–40 international working group in which nine laboratories participated, some who had previously detected SV–40, some who had not and some laboratories that were new to the field. This was a very tightly structured endeavor, highly collaborative and some were very unhappy with the result in that those who had previously detected SV–40 were unable to do so in the study that they collaborated in and that we all collaborated in.

We endorsed the research recommendations of the IOM, of which there were five. The second of those has to do with development of sensitive specific and standardized tests for detection of SV–40 DNA. SV–40 DNA PCR is a highly powerful but difficult to standardize procedure and similar issues came up with other PCR assays with previous agents, be it hepatitis C or HIV and the like. The first recommendation was actually this antibody test kind of thing, and we endorse that and we are working with other university laboratories on that. With those technologies, I think that the third and fourth and fifth recommendations can be implemented, which has to do with the evaluation of people and specimens prior to 1955 to evaluate current populations in terms of transmission and to advance the question of the vaccine recipients. And I think the weakness that the chairman was mentioning has to do with the lack of perfection. We can be very highly confident with respect to the exposure of the vaccine recipients, but having a blood test would be helpful.

Ms. WATSON. I'm thinking prospectively, and I know that the field of science is always evolving, and I would think 1997's results are not conclusive because we are hearing to the contrary. So what I would like to hear, and maybe you're not prepared to even comment, is how could we plan a research agenda that would use specific serologic tests for SV–40, and maybe you're not prepared to address that. But I would like to see us use probing minds because there's too much, as I would think now, inaccuracies, and too much conflict as it addresses the results of various studies. And so to take it out of the realm of speculation and this confusion, I would like to see you come up with a new research strategy that all of you collaborate on for 2000 and beyond. Well, let's say 2003 and beyond.

If you're not ready to respond to that, I can understand, but I'm throwing out a recommendation. I'm just hearing from too many people. I understand there are some parents that either have testified or will testify and I think as scientists we ought to continue to research so that we could once and for all make conclusions that will hold.

Thank you, Mr. Chairman.

Mr. BURTON. Let me just followup. What I would like to do because we're going to be running short of time. We're going to have more votes. Could we submit to you questions for the record to be answered by you and sent back so we can review them?

Dr. GOEDERT. Certainly.

Mr. BURTON. OK. Well, then we'll do that. Let me just ask, followup on what the Congresswoman just said. You know, there were 60 laboratories that conducted tests that showed a contrary result. We have scientists around the world, eminent scientists that disagree with the results that you folks base your findings on, and many of these scientists are every bit as eminent if not more eminent than Dr. Strickler—is it Strickler or Stricker? Stricker I guess it is—who as I stated earlier was working for you. When you're following up on what Representative Watson suggested, would it be possible for you to contact those scientific laboratories and those scientists who had contrary results to take a look at their findings to find out if there's something that you missed, and we would be very happy to give you the names of those laboratories as well as the scientists involved so that you wouldn't rely just on what you folks found, but also what these other laboratories and eminent scientists found. Would you be willing to do that?

Dr. GOEDERT. Certainly, Mr. Chairman. The nine laboratory study that we did included laboratories, the preeminent ones that had previously found positive results. They did not when they——

Mr. BURTON. You said nine. There were 60. How come you didn't talk to the other 51?

Dr. GOEDERT. Well, some—I'll be happy to if you send me the names of the other ones.

Mr. BURTON. We'll send that to you.

And the other things I'd just like to conclude with is that many Congressmen and Congresswomen—and I'm not speaking for Congresswoman Watson, I'm speaking for myself—are a little bit suspicious of some of the results of tests and other things that we've seen coming out of FDA and HHS, and I'm not pointing this at you, Doctor, or Doctors. But we have seen the results that came back that show results that are unbelievable. And we've been stonewalled on other issues where there might be lawsuits filed against pharmaceutical companies that have had research projects that have worked with, I think, with our health agencies. And so we're just a little bit suspicious of those things. That's why when we hear these results, and I hope you—if you wouldn't mind, I hope you'll stick around a little bit and hear some of the information from these parents and other scientists. I think Dr. Gazdar is here, I think he's going to testify. I think he was on the other side of this issue at one time. I wish you could just listen to what they have to say and maybe that would illuminate the issue a little bit more and maybe help in getting to the bottom of this.

Dr. GOEDERT. I'll be happy to do whatever I can.

Mr. BURTON. Thank you sir, very much. Any other comments?

Ms. WATSON. Just before you step away from this panel I would just like to thank you for being here, and I want all of you to keep your minds open and I think that our environment, and I'm talking about comprehensive environment, is so full today with contaminants. It indeed is affecting our health to the point that there are new mutations and I'm concerned about this. More people are coming up with cancer, and we must look at everything that we spray into our environment, that we put on our soil, that we ingest, that we use intravenously.

And so I don't want closed minds. We can't depend on research that was done years ago. We must think about our future and what we might contribute to it. So I would hope that you would agree just to keep flexible and we certainly understand and we know the shortfalls of money and we know where our focus is. But we would support you in coming up with a strategy for new studies. We will give you guidance and direction, I'm sure from the standpoint of this committee, as to what we'd like to see. And we'll even work for the funding. So blue sky, if you will. I used to say that to bureaucrats. You know, if you had all that you needed, what would you like? And I tell you they were in such little tight boxes they couldn't even—blue sky. So we're giving you such opportunity with our support to take another look and work in a collaborative way to save our people and particularly our children.

Thank you so much.

Mr. BURTON. We will get you the names of the laboratories and the names of these other eminent scientists who have differing views and hopefully you can followup with them and cross-check their results with the results you've had and maybe additional studies, as Representative Watson suggested, would be done to make sure that we get to the bottom of this. In any event, I hope you'll stick around just a little bit and hear what these other folks have to say. It might be illuminating. Thank you very much.

Our next panel is my good friend Barbara Loe Fisher. She's the cofounder and president of the National Vaccine Information Center. Ms Eileen Grabinski, she's the mother of an injured child. Mr. Stanley Kops, he's an attorney from Pennsylvania, and Dr. Gazdar, whom I mentioned a few moments ago, who's a therapeutic oncology professor, I guess professor, at the University of Texas Southwestern Oncology in Dallas.

Would you all please stand and raise your right hands?

[Witnesses sworn.]

Mr. BURTON. As I said to the first panel, because we are going to have a whole bunch of votes I would like to try to keep the testimony to 5 minutes for each one of you so we can get to the questions, which I think might be a little bit more illuminating, and let's just go right down the line.

OK, we'll start with Ms. Fisher. I don't know what the reason is for that but evidently you have more influence with John than anybody else. Go ahead.

STATEMENTS OF BARBARA LOE FISHER, PRESIDENT, NATIONAL VACCINE INFORMATION CENTER; EILEEN GRABINSKI, MOTHER OF AN INJURED CHILD; STANLEY P. KOPS, ESQ., ATTORNEY AT LAW; AND ADI GAZDAR, PH.D., UNIVERSITY OF TEXAS SOUTHWESTERN ONCOLOGY, HAMON CENTER FOR THERAPEUTIC ONCOLOGY

Ms. FISHER. My name is Barbara Loe Fisher. I'm the mother of a DPT vaccine injured son and cofounder and president of the National Vaccine Information Center. I've spent the last 21 years working with other participants to prevent vaccine injuries and deaths through public education.

The story you're about to hear involves a pharmaceutical company which used monkeys to make polio vaccine, government health agencies responsible for making sure the vaccine was not contaminated with monkey viruses, and individuals who are now dying from cancerous tumors that contain a monkey virus which appears to have contaminated that polio vaccine. At the heart of this story is a violation of the public trust and the informed consent ethic.

I began speaking and writing about monkey virus contamination of polio vaccines 10 years ago when questions were raised in the medical literature about whether the use of monkeys infected with monkey viruses to produce oral polio vaccines was responsible for HIV and the AIDS epidemic. Between 1994 and 1997 I submitted several Freedom of Information Act requests to the government regarding testing of certain lots of oral polio vaccine for monkey virus contamination. It was in 1960 that a NIH scientist named Bernice Eddy discovered that rhesus monkey kidney cells used to make the Salk polio vaccine and experimental oral polio vaccines could cause cancer when injected into lab animals.

Later that year the cancer causing virus in the rhesus monkey kidney cells was identified as SV–40, or Simian Virus 40, the 40th monkey virus to be discovered. Sadly, though, the American people were not told the truth about this in 1960. The SV–40 contaminated stocks of Salk polo vaccine were never withdrawn from the market, but continued to be given to American children until early 1963 with full knowledge of Federal health agencies.

At a conference on SV–40 and human cancers held by the National Institutes of Health in 1997 there was no disagreement among both government and nongovernment scientists about this fact. The only disagreement was whether SV–40 was actually being identified in the cancerous tumors of children and adults alive today and, if it was, whether the monkey virus was in fact responsible for their cancer. Nongovernment scientists working in independent labs around the world said yes. But the scientists connected with the U.S. Government said no.

As you have already pointed out, Mr. Chairman, the Institute of Medicine and highly credentialed nongovernment scientists in multiple labs around the world continue to identify SV–40 in human brain and lung cancers of children and adults and are finding that SV–40 is also associated with bone cancers and non-Hodgkins lymphomas. The majority of these independent scientists have concluded that, yes, SV–40 does cause human cancers.

Up until this hearing to date the world scientific community has assumed that the only polio vaccine that was contaminated with SV–40 and released for use by millions of Americans was Jonas Salk's killed polio vaccine, which stopped being used in 1963 because it was replaced by Albert Sabin's live polio vaccine. Why? Because the oral polio vaccine manufacturer and Federal health agencies have told everyone that while the Salk vaccine was made using the SV–40 infected rhesus monkey kidney tissues after 1963 the oral polio vaccine was made using African Green monkeys, which are rarely infected with SV–40. The vaccine manufacturer and government officials have insisted that the switch from rhesus monkeys to African Green as well as testing protocols to detect SV–40 prevented SV–40 from contaminating oral polio vaccine after 1963.

However, you will be presented with evidence today that suggests, one, the original seed stocks of oral polio vaccine were made using the rhesus monkey and were contaminated with SV–40; two, the major oral polio vaccine manufacturer did not adequately test their master seed stocks which reportedly contained SV–40 but used them to produce vaccine released for use by American children from the 1960's through the 1990's; and, three, Federal regulatory agencies either did not know or knew and did not do anything about evidence that SV–40 contaminated oral polio vaccine was released for use by the public from the 1960's to the 1990's.

If SV–40 contaminated rhesus monkeys were used to produce original oral polio vaccine stocks, and if these seed stocks were used to produce oral polio vaccine that was swallowed by American children through the 1990's, and if SV–40 does cause human brain, lung and bone cancers, then this could explain why children today, who were not born before 1963 and never got SV–40 contaminated Salk vaccines, are now sick and dying from cancerous tumors containing DNA from a monkey virus that was in those vaccines. Pediatric brain cancer, once rare, rose during the past few decades, according to the National Cancer Institute. But we don't know how many of these children had or have SV–40 in their brain tumors because nobody checks, how many of these children are sick and dying because the manufacturer of oral polio vaccine did not follow the rules and government health agencies did not enforce the rules.

Since 1999, the United States has discontinued use of the live oral polio vaccine and American children are now getting a killed vaccine that is reportedly SV–40 free. So why is it important today to find out whether or not the oral vaccine used to eradicate polio was in fact contaminated with the cancer causing monkey virus and that the vaccine manufacturer knew it and government health agencies looked the other way?

It is important because if it's true, then a precedent has been set and that precedent may well be affecting decisions being made by government health agencies today about what kinds of animal tissue cultures vaccine manufacturers will be allowed to use to make new vaccines and what kinds of tests will be required to ensure that the vaccines do not contain animal viruses or other contaminants.

I've just ended a 4-year term as the consumer voting member of the FDA Vaccines and Related Biological Products Advisory Committee. My service on that committee gave me a new appreciation for the dedicated work of a number of fine scientists employed by the FDA who take their regulatory duties very seriously and are working hard to regulate the vaccine industry with very limited resources and limited support within and outside of the government. But there are legitimate concerns which I and others have voiced in the past and continue to have about whether government standards for requiring vaccine manufacturers to prove the safety and efficacy of vaccines are high enough and whether the tests used by the manufacturers and the government to ensure the safety of vaccines are good enough.

I urge this committee and other congressional committees to carefully review the transcripts of meetings of the FDA Vaccines and Related Biological Products Advisory Committee, specifically those which were held in 1998, 2000 and 2001 and dealt with adventitious agent contamination of vaccines. Vaccine manufacturers are asking the FDA for permission to use cells from human and animal cancer tumors; that is, cancer cells, to make HIV and other viral vaccines in the future that would be used on a mass basis by the American population. There has been a Federal ban on the use of cancer cells to produce vaccines since 1954. But active consideration is now being given to lift that ban despite the acknowledged risks of contamination with adventitious agents, including residual DNA and RNA.

There is frank admission that the limitations of technology and lack of scientific knowledge means there can be no guarantee that vaccines will not be contaminated with substances that could prove harmful to humans 1 day. Nevertheless, there are discussions about creating allowable thresholds for adventitious agent contamination of vaccines being made out of cancer cells that could contain residual DNA and RNA.

I don't think Congress or the public understands any of this. There should be a much wider discussion in the larger scientific community outside of Federal health agencies and the pharmaceutical industry as well as in Congress and by the public at large before decisions are made to proceed with producing vaccines that use cancer cells and have legally allowable thresholds of adventitious agent contamination.

Mr. BURTON. Ms. Fisher.

Ms. FISHER. I know. I'll wrap up here.

Mr. BURTON. Well, you can submit the rest of it for the record, but what I'd like to say is that those hearings that you were a part of——

Ms. FISHER. I was on the committee.

Mr. BURTON. I would like for you to give us copies of those transcripts if you could.

Ms. FISHER. I have.

Mr. BURTON. OK. And with that can you submit the rest of it for the record?

Ms. FISHER. I will. I just would like to thank you Chairman Burton for everything you've done to hold these hearings in the past 2 years, so that we can have a safer vaccine system.

[The prepared statement of Ms. Fisher follows:]

Oral Presentation
Barbara Loe Fisher
Co-founder & President
National Vaccine Information Center
September 10, 2003
Subcommittee on Human Rights and Wellness
U.S. House Government Reform Committee
U.S. House of Representatives, Washington, D.C.

"The SV40 Virus: Has Tainted Polio Vaccine Caused An Increase in Cancer?"

My name is Barbara Loe Fisher and I am the mother of a DPT-vaccine injured son and the co-founder and president of the National Vaccine Information Center. I have spent the last 21 years working with other parents to prevent vaccine injuries and deaths through public education and defending the right to exercise informed consent to vaccination. (Coulter HL, Fisher BL. 1985. *DPT: A Shot in the Dark.* New York: Harcourt Brace Jovanovich.; Attachment 1 – Allen A. May 6, 2001. A Shot in the Dark. *New York Times Magazine*; Konrad W. and Ginsburg EH. June-July 2000. Who's Calling the Shots? *Offspring Magazine.*)

The shocking story you are about to hear involves a pharmaceutical company which used monkeys to make polio vaccine, government health agencies responsible for making sure the vaccine was not contaminated with monkey viruses, and individuals who are now are dying from cancerous tumors that contain a monkey virus which appears to have contaminated that polio vaccine. At the heart of this tragic story is a violation of the public trust and the informed consent ethic. It is a story about what happens when the legal and moral duty for industry and government to insure that a vaccine will not harm individuals is sacrificed to insure acceptance and mass use of a vaccine by the entire population. It shows what can happen when Congress, which has oversight authority over federal health agencies, blindly trusts and fails to verify.

I began speaking and writing about monkey virus contamination of polio vaccines ten years ago when questions were raised in the medical literature about whether the use of monkeys infected with monkey viruses to produce oral polio vaccines was responsible for HIV and the AIDS epidemic. (Attachment 2: Kyle, W.S. 1992. Simian retroviruses, poliovaccine, and origin of AIDS. *The Lancet* 339: 600-601.) Between 1994 and 1997 I submitted several Freedom of Information Act (FOIA) requests to the government regarding testing of certain lots of oral polio vaccine for monkey virus contamination (Attachment 3 – Correspondence between BL Fisher and FDA) During the course of my research I discovered that it was well known that the first polio vaccine produced in the 1950's – the inactivated polio vaccine created by Jonas Salk – was made using rhesus monkeys that were infected with a monkey virus called simian virus 40 or SV40.

It was in 1960 that an NIH scientist named Bernice Eddy discovered that rhesus monkey kidney cells used to make the Salk polio vaccine and experimental oral polio vaccines could cause cancer when injected into lab animals. Later that year the cancer-causing virus in the rhesus monkey kidney cells was identified as SV40 or simian virus 40, the 40th monkey virus to be discovered. (Shorter, e. 1987. *The Health Century)*

Sadly, the American people were not told the truth about this in 1960. The SV40 contaminated stocks of Salk polio vaccine were never withdrawn from the market but continued to be given to American children until early 1963 with full knowledge of federal health agencies.

Between 1955 and early 1963, nearly 100 million American children had been given polio vaccine contaminated with the monkey virus, SV40. (Institute of Medicine, National Academy of Sciences . 2002. *Immunization Safety Review: SV40 Contamination of Polio Vaccine and Cancer.* Washington, D.C.: National Academy Press)

Today, U.S. federal health agencies admit the following two facts:

1. Salk polio vaccine released for public use between 1955 and 1963 was contaminated with SV40; and
2. SV40 has been proven to cause cancer in animals.

In fact, at a conference on SV-40 and human cancers held by the National Institutes of Health in 1997, which I attended, there was no disagreement among both government and non-government scientists about these two facts. The only disagreement was whether SV40 was actually being identified in the cancerous tumors of children and adults alive today and, if it was, whether the monkey virus was in fact responsible for their cancer. Non-government scientists working in independent labs around the world said, "Yes." But the scientists connected with the U.S. government said "No." (Transcript of FDA, CDC, NIH, NIP, NVPO January 27-28, 1997 *Workshop on Simian Virus 40: A Possible Human Polyomavirus*).

Today, there are scientists associated with the US government who continue to deny that SV40 causes human cancer or that SV40 associated cancers have had any effect on cancer rates since the early 1960's. However, highly credentialed non-government scientists in multiple labs around the world continue to identify SV40 in human brain and lung cancers of children and adults and are finding that SV40 is also associated with bone cancers and Non-Hodgkin's Lymphomas. The majority of these independent scientists have concluded that, yes, SV40 does cause human cancers. (Attachment 4 – Gazdar AE, Butel JS, Carbone M. 2002. SV40 and human tumours: myth, association or causality*? Nature* 2: 957-964)

And in a report published in 2001, the Institute of Medicine Immunization Safety Review Committee stated that "in light of the biological evidence supporting the theory that SV40 contamination of polio vaccines could contribute to human cancers, the Committee recommends continued public health attention in the form of policy analysis, communication and targeted biological research."

Up until this hearing today, the world scientific community has assumed that the only polio vaccine that was contaminated with SV40 and released for use by millions of Americans was Jonas Salk's killed polio vaccine, which stopped being used in 1963 because it was replaced by Albert Sabin's live oral polio vaccine. Why? Because the oral polio vaccine manufacturer and federal health agencies have told everyone that while the Salk vaccine was made using the SV40 infected rhesus monkey kidney tissues, after 1963 the oral polio vaccine was made using African Green monkeys, which are rarely infected with SV40. The vaccine manufacturer and government officials have insisted that the switch from rhesus monkey to African Green as well as testing protocols to detect SV40 prevented SV40 from contaminating oral polio vaccine after 1963. (Attachment 5: Statement of Bonnie Brock, Lederle, at Jan. 27-28, 1997 Workshop on SV40, transcript pages 300-307).

However, you will be presented with evidence today that suggests (Attachment 6: Kops SP. 2000. Oral polio vaccine and human cancer: a reassessment of SV40 as a contaminant based upon legal documents. *Anticancer Research* 20: 4745-4749. and Oral Testimony, Stanley Kops, Esq. Subcommittee on Human Rights and Wellness, US Government Reform Committee, September 10, 2003):

1. the original seed stocks of oral polio vaccine were made using the rhesus monkey and were contaminated with SV40;

2. the major oral polio vaccine manufacturer did not adequately test their master seed stocks which reportedly contained SV40 but used them to produce vaccine released for use by American children from the 1960's through the 1990's;
3. Federal regulatory agencies either did not know or knew and did not do anything about evidence that SV40 contaminated oral polio vaccine was released for use by the public from the 1960's through the 1990's;

If SV40 contaminated rhesus monkeys were used to produce original oral polio vaccine seed stocks, and if these seed stocks were used to produce oral polio vaccine that was swallowed by American children through the 1990's, and if SV40 does cause human brain, lung and bone cancers, then this could explain why children today, who were not born before 1963 and never got the SV40 contaminated Salk vaccines, are now sick and dying from cancerous tumors containing DNA from a monkey virus that was in those vaccines. Pediatric brain cancer, once rare, rose during the past few decades according to the National Cancer Institute. But we don't know how many of these children had or have SV40 in their brain tumors because nobody checks. How many of these children are sick and dying because the manufacturer of oral polio vaccine did not follow the rules and government health agencies didn't enforce the rules?

Since 1999, the US has discontinued use of the live oral polio vaccine and American children are now getting a killed polio vaccine that is reportedly SV40 free. So why is it important today to find out whether or not the oral vaccine used to eradicate polio was in fact contaminated with a cancer causing monkey virus, and that the vaccine manufacturer knew it, and that government health agencies looked the other way?

It is important because if it is true, then a precedent has been set. And that precedent may well be affecting decisions being made by government health agencies today about what kinds of animal tissue cultures vaccine manufacturers will be allowed to use to make new vaccines and what kinds of tests will be required to insure that the vaccines do not contain animal viruses or other contaminants.

Drugs and vaccines are very different. Drugs are used to cure sick people while vaccines are required by law in this country to be given to healthy people, primarily children. The standards for proof of safety and efficacy of vaccines should be higher than for any other pharmaceutical product we use.

I have just ended a four year term as the consumer voting member of the FDA Vaccines and Related Biological Products Advisory Committee. My service on that committee gave me a new appreciation for the dedicated work of a number of fine scientists employed by the FDA, who take their regulatory duties very seriously and are working hard to regulate the vaccine industry with very limited resources and limited support within and outside of government.

However, there are legitimate concerns which I and others have voiced in the past and continue to have about whether government standards for requiring vaccine manufacturers to prove the safety and efficacy of vaccines are high enough and whether the tests used by the manufacturers and the government to insure the safety of vaccines are good enough. (Attachment 7: National investigative news reports, including Wechsler P. November 11, 1996. A Shot in the Dark. *New York Magazine.*; Rock, A. December 1996: The Lethal Dangers of the Billion Dollar Vaccine Business. *Money Magazine.;* Bookchin D, Schumacher J. June 1997. The Lonely Crusade of Walter Kyle*. Boston Magazine;* Bookchin D., Schumacher J. February 2000: The Virus and the Vaccine. *Atlantic Monthly Magazine.)*

I urge this Committee and other congressional committees to carefully review the transcripts of meetings of the FDA Vaccines and Related Biological Products Advisory Committee, specifically those which were held in 1998; 2000; 2001 and dealt with adventitious agent contamination of vaccines. Vaccine manufacturers are asking the FDA for permission to use cells from human and animal cancer tumors – that is cancer cells – to make HIV and other viral vaccines in the future that would be used on a mass basis by the American population. There has been a federal ban on use of cancer cells to produce vaccines since 1954 but active consideration is being given now to lift that ban despite the acknowledged risks of contamination with adventitious agents, including residual DNA and RNA.

.(Attachment 8: Excerpt from November 19, 1998 FDA Vaccines and Related Biological Products Advisory Committee meeting, transcript pages 29-52).

There is frank admission that the limitations of technology and lack of scientific knowledge means there can be no guarantee the vaccines will not be contaminated with substances that could prove harmful to humans one day. Nevertheless, there are plans to set allowable threshholds for adventitious agent contamination of vaccines being made out of cancer cells that would contain residual DNA and RNA. (Attachment 9: Excerpts from May 12, 2000 FDA Vaccines and Related Biological Products Advisory Committee meeting transcript and Attachment 10: Excerpts from May 16, 2001 FDA Vaccines and Related Biological Products Advisory Committee meeting transcript)

I do not think Congress or the public understands any of this. There should be a much wider discussion in the larger scientific community outside of federal health agencies and the pharmaceutical industry, as well as in Congress and by the public at large before decisions are made to proceed with producing vaccines that use cancer cells and have legally allowable threshholds of adventitious agent contamination.

Past is often prologue. So much can be learned from understanding the mistakes of the past so that the same mistakes are not made in the future.

Outstanding questions about the links between vaccines, government vaccine policies and the epidemic of chronic disease in our children, including autism, learning disabilities, ADHD, asthma, diabetes and, as we have discussed today, cancer are not going away. Questions about the links between vaccines that US military soldiers are required to take, including anthrax and smallpox vaccines, and the subsequent death or permanent health problems being suffered by those previously healthy, young recruits are not going away. They will never go away when the main defense of industry and government health officials is that when anything bad happens after vaccination it is just a coincidence. I can tell you, the American public, especially parents, are not buying it. And they shouldn't buy it, especially when the kind of evidence that you will hear today suggests official government and industry denials are simply a way of avoiding taking responsibility for failing to do everything they can to minimize the risks of vaccines.

We owe it to our children and grandchildren to do everything we can to find out the truth about vaccine risks and make the mass vaccination system as safe as it can be. I believe that can only be done if Congress exercises more oversight authority over federal health agencies responsible for vaccine research, development, regulation, policymaking, promotion and monitoring of vaccine side effects. Conflict of interest legislation is urgently needed to separate government health agencies from financial and other ties with the vaccine industry so that government health officials can be free to do the job they are supposed to do:

protect the health and well being of every American and not simply protect the vaccine supply. (Attachment 11: Investigative news report by UPI reporter Mark Benjamin. July 20, 2003. *Chicago Sun Times, Washington Times*)

Before I conclude, I would like to thank you, Chairman Burton, for all you have done during the past three years to investigate and bring to the attention of Congress and the American people the fact that our nation's mass vaccination system must be reformed to make it safer. You have had the courage to stand up for those who suffer greatly when a vaccine's risks turn out to be 100 percent for them or their child and you have done it against great opposition from powerful special interest groups with vested interests in protecting the status quo. Your tireless efforts on behalf of so many will not be in vain because the truth will shine bright and clear in the end no matter how long it takes.

Mr. BURTON. Thank you very much.

Ms. Grabinski.

Ms. GRABINSKI. Hi. How you doing?

Mr. BURTON. You have a child that you feel has been damaged by the vaccine?

Ms. GRABINSKI. Yes, I do and I brought him with me. He's sitting in the wheelchair there.

Mr. BURTON. Your son's in the wheelchair over there?

Ms. GRABINSKI. Yes.

Mr. BURTON. OK. Thank you. Mark. OK.

Ms. GRABINSKI. Right. His name is Mark Marino.

Mark was beautiful, healthy baby when he was born. He was growing up normally the way you would expect any normal child to grow up. He wasn't any different than my son Joe. He received routine care that babies get, including his vaccinations with the oral polio vaccine known as trivalent. Shortly before Mark's tumor was found by the doctor he was not acting right and I knew something was wrong.

Marks' tumor turned out to be a rare tumor. His hospital stays were nightmares every time he had to stay for surgery. We always had it in our minds he would never come out alive because the doctors told us it was a rare tumor. Mark had to have part of his skull removed to save his life, and now he has to wear a helmet every day for the rest of his life.

When Mark was born and when tests were done to see his intelligence, they were pretty good but after his operations they deteriorated and now he has limited ability. This limitation lasted from age 5 to now. When he was 5 I was told he was functioning somewhere between 3 and 5 years old. Nothing has changed since then.

Mark loves to paint, draw and to go out with other people, but we cannot go out often because he is in danger of having epileptic occurrence. Since the first surgery Mark has been a toddler. He never grew up. He rarely participates in family functions and when he does he has to be constantly supervised.

I try to keep him busy because he's with me 24 hours a day. He can do simple chores. He can mix the salads for dinner, sweep the kitchen floor on his knees. He thinks he cleaned the whole house. He can put away the cans after shopping. He's so proud of himself after he does the chores it's the biggest thrill of the day for him. He talks to his stuffed animals. They are his friends who he can count on being there for him every day. He takes them almost everywhere he goes.

He watches TV, but only cartoons. In his mind he believes that 1 day he will be in a cartoon. He gives his painting and coloring pages to people he meets to show them he loves them and he thinks they love him also. You know they love him back. He paints rocks and sea shells or anything that he can paint gold. The pirates in his cartoons hunt for gold, so he hunts for gold. The only difference is he gives his gold away.

He says his prayers at night and has a picture of God on his wall. He knows that God is his friend and the only one who can help him. And he never loses his faith. He is convinced that God hears him and will help him. We have to learn every day how to cope with every aspect of his life.

I have never been bitter about my son's condition until recently. Because I cannot go out a lot, I spend a lot of time on the Internet. On one of the Internet searches, I found out about there was an issue of SV–40 and childhood tumors. Eventually I found out that Mark's tumorous material was available at the hospital where he was treated. The materials were tested, and I was advised that the SV–40 was found in his tumor. What I thought was an act of God I know now was what—I'm sorry, I'm a little nervous—I now learned was an act of man.

I am not a scientist or a lawyer; I'm just a mother, and I feel cheated and robbed out of my life, my son's life, our entire family's life by someone who'd use a childhood vaccine in an unsafe manner and allow my child, along with many other children, to be exposed to this virus. I can only hope that Mark's prayers to God will be answered by the scientists and maybe there is something that can be done to reverse his condition.

My reason for testifying here today is for two reasons: to tell the story of my brave son and to ask Congress to do whatever is necessary to protect children like my son from ever having to face what he has faced and from what our entire family has faced.

Thank you.

Mr. BURTON. Thank you, Ms. Grabinski. And I don't think there is anything that we could say that will help the situation, but you have our prayers and our gratitude for what you go through.

Ms. GRABINSKI. Thank you.

Mr. BURTON. Mr. Kops.

Mr. KOPS. Good afternoon.

I have represented and still represent individuals who have suffered injuries from the Orimune oral polio vaccine that was utilized in the United States from 1962 until 1999 when Orimune vaccine, oral vaccine, could no longer be sold in the United States for immunizations.

The history of this negligence of both the vaccine manufacturer and the government can be found in reported decisions. The Supreme Court in 1988 in a unanimous decision written by Justice Marshall, found that if the vaccine manufacturer and/or the regulator failed to look at the test results and failed to determine what those test results showed, the government did not have any permission to do so. They did not have the discretion to avoid that review. In fact, at oral argument, I believe it was Justice Scalia who asked the following question of the Solicitor General: Supposing the government did not make any examination of the application at all, or any determination other than some papers have been filed and now we will issue a license; would this comply with the regulatory system?

Counsel for the government: No, it would not comply with the regulation.

Question from Justice Scalia, I believe: It would violate a mandatory duty wouldn't it?

Counsel: In that extreme instance you are talking about, it would definitely violate the regulations.

That could be found both in the transcript of oral argument and at footnote 10 to the opinion.

What I am here to testify today is that's exactly what happened. They did not look, the regulators, and the vaccine manufacturer did not submit test results. This is a white-and-black situation. Either the test results exist and they can be produced, or they do not exist because they were either not performed or performed and the results were so horrendous that they would rather not submit the test results than submit those that prove the exact points that this committee is investigating.

There are three types of wild polio. Therefore, there was a need to create three different vaccines. The IPV, the killed vaccine, was always a trivalent product. As to the oral polio vaccine, they were first made as individual monovalent pools and then later combined as a trivalent vaccine.

Between 1964 and 1967, a single manufacturer in this country, Wyeth-Lederle had 84 percent of this market. In 1977 it had 100 percent of the market. Up until today, no scientist has had the complete data to challenge the assertions made by scientists and by the vaccine manufacturer. In fact, I heard today in the testimony of the head of the NIH cancer epidemiology session that all vaccines after 1963 did not contain SV–40. That is just wrong. They did contain SV–40 because there are test results that I have, which now the committee has, that show the positive vacuolating agent in released product. Those were the test results that were shown to the IOM, the Institutes of Medicine.

I have been lucky to have had the honor to represent people like Eileen and Mark and others, and during that representation when it was only about polio, I was given the actual test results of various products which show that they were positive for SV–40. You could see that in exhibit 21. The use of Rhesus monkeys, something that this vaccine manufacturer guaranteed the entire scientific world that it never used in manufacture, is in fact exactly what they used.

If you look at exhibit No. 11, there is a released monovalent pool of this manufacturer. It shows that the monkeys utilized were Rhesus. It shows in a subsequent exhibit, No. 13, that on January 15, 1990, American Cyanamid requested from the regulators permission to release five monovalent pools, all made in Rhesus monkeys. The pool numbers 263, 265, 283, 501, and 509. I see I'm over my time so——

Mr. BURTON. Can we get into this a little bit more, Mr. Kops, in the question section?

Mr. KOPS. Certainly.

Mr. BURTON. This is a pretty voluminous bit of information you sent from Lederle Laboratories, and I think we're going to have to digest this over a period of time, but we have some questions we'd like to ask you about that.

[The prepared statement of Mr. Kops follows:]

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ORAL PRESENTATION TO THE SUBCOMMITTEE ON HUMAN RIGHTS AND WELLNESS OF THE COMMITTEE ON GOVERNMENT REFORM: "THE SV40 VIRUS: HAS TAINTED POLIO VACCINE CAUSED AN INCREASE IN CANCER?"

I have represented, and am still representing, individuals who have been paralyzed, killed, and or severely damaged as a result of the Orimune vaccine manufactured, sold and distributed from 1962 until the year 2000. Though attorneys are advocates, and I am an advocate in the courtroom for my clients,, I have left my attorney's hat in Philadelphia and have come before this committee to raise questions as someone who has authored peer reviewed articles in the medical literature on the issue of SV40 and human cancers.

There is a history of negligence involving this vaccine manufacturer and the regulators, who are the employees of the Government agency charged with the responsibility of insuring the safety, purity and potency of vaccines. In 1988, the United States Supreme Court in a unanimous decision, (Berkovitz v. United States, 486 U.S. 531) held that under federal government FDA regulations, the vaccine manufacturer must conduct a variety of tests to measure the safety of vaccines at each stage of the manufacturing process. (Exhibit 1 - a copy of the regulations) Upon completion of the manufacturing process, and the required testing, the manufacturer is required to submit a license application to the FDA. In addition, the manufacturer must submit the data showing that the tests were performed and that the test passed all of the safety standards. A license can only be issued by the FDA upon a showing that the vaccine manufacturer has met the standards designed to insure the continued safety, purity and potency of the vaccine prescribed in the regulations. **Berkovitz** at p. 540-542.

In a question and answer at oral argument between one of the Justices of the Supreme Court and the Solicitor General, the following took place:

> Q. [Supposing the DBS] did not make any examination of the application at all, or any determination other than some papers have been filed and I will now issue the license. "Would that comply with the regulations?
>
> [Counsel]: No it would not comply with the regulation.
>
> Q. It would violate a mandatory duty..., wouldn't it
>
> [Counsel] In the extreme instance you are talking about..., it would definitely violate that regulation." Tr. of Oral Arg. 34-35.

Berkovitz, 486 U.S. at 544 n.10.

That extreme case is exactly what happened with the Sabin Oral Poliovirus known as Orimune manufactured by Lederle Laboratories owned by American Cyanamid, and today known as Wyeth Lederle – the vaccine safety tests were not submitted, the regulators did not look, and infants in the United States became paralyzed, or died, and there are now clear instances of cancer reported in the children and individuals who received this product.

Since there are three types of wild poliovirus, there was a need to create three vaccines. Trivalent polio vaccine contained all three types combined. This manufacturer was the first to combine all three polio types into a single live oral polio vaccine known as OPV. Between 1964 and 1977, Lederle had become the largest manufacturer of OPV in the United States and claimed to have 84% of the market. After 1977, it was the sole manufacturer until the vaccine was no longer sold in the United States. See FDA Docket No. 86N-0027.

Up until today, no scientist has had the complete data to challenge all of the assertions made by this vaccine manufacturer. Wyeth/Lederle has demanded in every lawsuit that all documents be marked confidential and could not be revealed to the scientific world. The documents that you will be seeing today are not the full complement of the documents which will tell a frightening story of contamination of live oral polio vaccine with a monkey virus known as SV40 – they are documents not under seal which were collected, from other litigation across the country, from the United States of America during litigation, and from litigation with the vaccine manufacturer in cases involving victims of this oral polio vaccine.

Within weeks of being licensed, in 1962, American Cyanamid made the following two promises to the consuming public, the scientific world, and the medical profession:

> Orimune 1, 2, and 3 are produced and tested in accordance with the regulations of the United States Public Health Service for production of Poliovirus Vaccine, Live, Oral. The manufacturer makes no representation or warranty, express or implied, with respect to the merchantability or fitness for use of these vaccines other than that they have been produced in accordance with the standards for their production prescribed by the United States Public Health Service and applicable thereto at the time of their release by the manufacturer.
>
> Can Oral Polio vaccine Cause Other Diseases?
> The safety regulations discussed above include many general safety tests. There are also tests designed especially to detect certain potentially pathogenic organisms, such as the vacuolating agent (SV40). Every production pool of Orimune is carefully tested many times for SV40, and any lot in which it is found is rejected.

(Exhibit 2)
Both of these claims were, and are false.

Today it is my intent to present evidence to prove that these statements repeated and reaffirmed at the presentation of Wyeth/Lederle at the SV40 conference, held in January 1997 under the auspices of the FDA and the N.I.H., were factually incorrect and intentionally misleading so as to keep the scientific world from conducting a thorough investigation.

This vaccine manufacturer, when it presented at the 1997 conference, and when it thereafter published its presentation in scientific journals, knew that the entire scientific world, which were all involved in a debate about SV40, would rely, as they had in the past, on their statements as to the safety of this product and that it could not possibly have contained the vacuolating agent SV40.

American Cyanamid, in both its presentation and, thereafter, in a scientific publication, has stated the following: "The cell culture substrate is prepared in primary monkey kidney cells obtained from green monkeys that do not harbor SV40 virus." (Exhibit 3) That statement was and is false. The only known manufacturer in the world who did not do blood tests on their monkeys to determine the presence or absence of SV40 is this manufacturer. In a sworn deposition given on October 2, 2002, the now head of the biological quality control, stated the following:

> Q. Did you ever see a serological test conducted to determine whether or not a African Green monkey was infected with SV40?
> A. I may have done – it may have been done for investigative purposes only.
> Q. Once or twice?
> A. Yeah, a couple times.
> Q. But it was never done as a matter of routine; is that correct/
> A Correct.

(Exhibit 4)

The vaccine manufacturer assured the scientific world that a single appearance of an adventitious agent such as SV40 at any stage of testing, results in the rejection of the cell batch. This statement was repeated from 1962 through 1999, and is now being stated in various courtrooms where litigation has been commenced against this vaccine manufacturer. As an example, in a Request for Admissions filed in a lawsuit now pending, this vaccine manufacturer answered a Request for Admission with the following assurance:

> REQUEST FOR ADMISSION NO. 33:
> American Cyanamid cannot insure if the alleged neutralization for SV40 from the Sabin Original Merck material Type II was successful.
> RESPONSE:
> Defendants object to this Request as it assumes SV40 was present in the Sabin Original Merck material Type II, an unproven conclusion. Expressly reserving, and without waiving their objections, and subject to them, defendants respond that they are not insurers but that they neutralized Sabin Original Merck Type II and tested it for the presence of adventitious agents. These tests were negative and subsequent testing on vaccine produced using the progeny of SOM Type II have been negative for SV40.

The internal standard operating procedures for this company do not provide for automatic rejection when an adventitious agent even when SV40 is found in the test results. This vaccine manufacture engages in repeat testing, and only in the extreme case where five failures in a row are recorded do they reject the harvest;; anything short of this is eventually tested into compliance. (Exhibit 5 - As of 1983 where two failures were permitted, which was modified as time progressed.)

Everyone in the scientific world has assumed that, from 1960 onward, once SV40 had been discovered in the Rhesus monkey kidney tissues utilized to produce oral polio vaccine, that Rhesus monkeys were abandoned and African Green monkeys would be utilized. The rate of SV40, and other adventitious agents, in Rhesus monkeys in the hands of this vaccine manufacturer is somewhere between 50-60% of all monkeys rejected. (*Results of Testing Production Lots of Oral Poliovirus Vaccine*, Exhibit 6) According to the vaccine manufacturer's internal records, African Green monkeys had about a 10% chance of being infected with SV40. (Exhibit 7) It also notes three different harvests, each of which showed the vacuolating agent, and this material was distributed throughout the United States.

In an authoritative text *Oncogenic Viruses*, Third Edition, by Ludwik Gross, M.D. F.A.C.P., he advised that the rate of Rhesus monkeys being infected with SV40 was at a much higher percentage. *See* Chapter 23, "Oncogenic Potency of Simian Virus 40 (SV40)," p. 829-887. (Exhibit 8)

In 1997, this vaccine manufacturer, through its presentation both orally and in writing, reconfirmed to the entire world what it had previously advised that: "All subsequent working seed strains have been prepared in CMK cells [African Green monkey] and screened to assure they are free of SV40 virus." That statement is utterly false. At no time did the regulators correct this misstatement. The master seeds were only prepared in Rhesus monkey kidneys, and as Dr. Sabin stated: "The three types of the large lots produced by Merck Sharp & Dohme in Rhesus monkey kidney cell cultures contained SV40." *WHO* Report 1969. (Exhibit 9)

The production seeds utilized by this vaccine manufacturer for the Type I and Type II components, of both the monovalent and trivalent vaccine utilized from at least 1960 until the beginning of the 1980s, were prepared only in Rhesus monkey kidney tissue and not African Green monkey tissue. (Exhibit 10) The following seeds were prepared in the Rhesus monkey kidney tissue: 3101, 3102, 3107, 2107, 45B52, 1102, and 45B51.

Not only were Rhesus monkeys utilized to produce seeds, they were utilized in the 1980s and early 1990s to produce the actual oral polio vaccine that was administered to millions of American children who were compelled to take that vaccine by state law, and were encouraged to take that vaccine by the federal regulators, by the vaccine manufacturer, by the Centers for Disease Control, and by an uninformed and misled Academy of Pediatrics. (Exhibit 11)

In litigation involving a young girl (the daughter of a Lieutenant Commander in the US Navy), the vaccine manufacturer claimed in August 2003 that after reasonable inquiry, and based upon the information presently known, or readily obtainable, they cannot admit or deny whether Rhesus monkey kidneys were even used to produce their seeds. (Exhibit 12)

This vaccine manufacturer claims that they are continuing to engage in a search to determine the species of the monkeys used to manufacture their seeds. For the ease of the Committee, and for this manufacturer who has been searching for these records for nearly a decade, I have submitted the monkey records showing that each single monkey used to make their seeds in the 1960s through the 1970s was a Rhesus monkey. The documents which I have submitted are documents that came out of the files of this vaccine manufacturer. The audacity to claim that it does not know what it used, in and of itself, is reprehensible. One can only question how this vaccine manufacturer was able to fool the supervising regulators for over 40 years. The utilization of rhesus monkeys kidney tissues that were prepared in the 1980s and utilized in the 1990s with the blessing of the regulators is administered in doses of vaccine into the mid-1990s. (Exhibit 13)

In a letter authored by Merck & Company to the Surgeon of the United States, Merck & Company admitted that their seeds contained SV40 and, therefore, they could not assure the Surgeon General that it could be safety removed (completely removed); therefore, they refused to produce the vaccine for commercial use. Dr. Sabin in correspondence directly with this vaccine manufacturer stated that he could not be sure that his original seed material for the Sabin Original Type III was free of SV40. (Exhibits 14 and 15)

By the year 1969, everyone knew from the reported literature, that all of the Merck Sharp & Dohme seeds contained SV40. This historical fact again was repeated in 1973 in a report given by Dr. Sabin.

As to the seeds that were utilized between the 1980s and up until 1999, those monkeys, especially for the Type I and Type II components, were monkeys that had been subject to a prior experimental test. The regulation is clear that experimental monkeys cannot be utilized as the source of material for production, let alone for production of a seed.

> Monkeys that have been used previously for experimental purposes shall not be used as a source of kidney tissue in the manufacture of vaccine.

(Exhibit 1)

This vaccine manufacturer in litigation is still searching for the test results of its master seeds, the test results of the initial seeds, and the test results showing the successful neutralization required by the safety regulations. This vaccine manufacturer is still looking for the serological test data. It is still looking for the waiver that they claim to have sought and received from the Government to use experimental monkeys.

The government of the United States of America – the FDA - has been searching for the same records, and amazingly cannot find records for twenty-two out of a total of twenty-six seeds which were utilized in the manufacture of Orimune from 1960 through 1999. The seeds that cannot be found are the master seeds, production seeds that were utilized between 1961 and 1980, the master seeds and intermediate seeds utilized between 1980 until 1999. The only reason why I believe neither the regulator nor the manufacturer can find the test results, is because they do not have all the safety tests required by the regulations and by the Supreme Court in **Berkovitz v. United States**.

When questions were asked by another regulatory agency, the government of Australia, as to the seed testing results for the master seeds, the technical superintendent of polio production of this vaccine manufacturer responded as follows:

> It should be made clear that Lederle did not test the original Sabin seeds for extraneous agents or neurovirulence since only 50 ml or less of each seed were provided by Dr. Sabin. It was presumed that if progeny of these seeds proved to be free of extraneous agents and have satisfactory neurovirulence the parent seeds were satisfactory.

(Exhibit 16)

Until the year 2000, no one published in peer reviewed journals any challenge to the promises and assurances of this company that it fully complied with the FDA's regulatory system. Everyone assumed that the vaccine manufacturer, and the regulators, fully implemented the regulatory standards.

In a memo authored by Dr. I. S. Danielson, the responsible head of this vaccine manufacturer, he noted in a discussion with a physician from Wilmington, Delaware, the following:

> He [the physician] stated that we do not say in our package circular that our vaccine is free of SV40 while Pfizer's does. I said that I had not even thought of this because the regulations require that demonstrable SV40 cannot be present; therefore, it went without saying that our vaccine is SV40 free.

(Exhibit 17)

Having been involved in litigation concerning the safety of Orimune, I knew that the statements made were false. I submitted an article which was peer reviewed by doctors and was published in *Anticancer Research* in December 2000 to bring his issue to the attention of the scientific world. (Exhibit 18) Since that time, not a single FDA regulator, or the vaccine manufacturer's employees have proven that any of the contentions raised in that article were erroneous. (Exhibit 19)

In July 2002, I was given the honor of presenting to the IOM a powerpoint documentary presentation showing the presence of SV40 in released products of this manufacturer, a copy of which is available to the members of the committee. The IOM wrote in its report, in October 2002, the following:

> Claims have been made that some oral polio vaccines might have been contaminated after 1963 (Kops 2000). The committee urges that FDA or other agencies address these claims to try to resolve the uncertainty regarding the possibility of exposure to SV40 after 1963. Appropriate assumptions about exposure are essential for conducting valid epidemiologic analyses of the risks that might be associated with contaminated OPV.

(Exhibits 20 and 21)

How has the vaccine manufacturer and the regulator responded to this demand of the IOM – with silence, no testing and no interest in revealing to the scientific world the truth.

Only Congress can correct and reaffirm the statutory command of the Vaccine Public Health Act, which was passed in 1902 to protect children who were compelled by state law to be vaccinated. The duty was to **insure** the safety, purity and potency of the vaccine. Congress in 1902 understood what the word **insure** meant - Congress in the year 2003 understands what the world **insure** means. The only ones who do not understand it, and who are disregarding this mandate, the law of the land, as enacted by Congress, are a vaccine manufacturer interested solely in profits, and the regulator who was complicit in numerous violations of the safety regulations not only as to SV40, but in every aspect of the safety of the Orimune vaccine.

If any of my statements are inaccurate, I invite the representatives of the regulators, and/or the vaccine manufacturer, to present their evidence that supports compliance, and to present the documents which support their assertions.

I thank you for inviting me to discuss this issue and I will be happy to respond to any questions.

STANLEY KOPS - ATTY

- Oral polio-vaccines were required to be free of SV40 since 1961
- Injected polio-vaccines were required to be free of SV40 since 1961, but contaminated vaccines were sold until 1963
- During litigation against Lederle I obtained documents indicating that SV40 was present in their product well after 1961

MR. KOPS

- These documents indicate that the Lederle product was never freed from SV40
- That Lederle did not follow the mandatory Code of Federal Regulations
- That Lederle knowingly distributed SV40-contaminated vaccines
- That Lederle never tested whether the neutralization procedure worked

MR. KOPS

Interoffice Memorandum between Mr. S. S. Aiston, Technical Superintendent of Polio Production Lederle and Mr. W. P. Cekleniak, dated March 14, 1979, states the following:

"It should be made clear that Lederle did not test the original Sabin seeds for extraneous agents or neurovirulence since only 50 ml or less of each seed were provided by Dr. Sabin. It was presumed that if progeny of these seeds proved to be free of extraneous agents and have satisfactory neurovirulence the parent seeds were satisfactory".

MR KOPS

- To prevent contamination manufacturers stated that all working seeds were prepared in SV40-free green monkeys
- Their own test showed that 10% of those monkeys were infected with SV40
- Furthermore, Rhesus monkeys were used to prepare type I and II seeds from 1961-1980.

MR. KOPS

- Seeds were prepared in rhesus kidney tissue and not African green monkey tissue for type I and II. This increases the risk of contamination
- 10% of green monkeys were SV40 infected
- Seeds were not tested for SV40
- Some seeds were not neutralized for SV40
- Seeds neutralized were not tested to see if the neutralization worked

MR. KOPS

- SV40 contamination was detected in all 3 monovalent types by Lederle
- Lederle ignored contamination and proceeded to release contaminated vaccines
- This failure to follow regulations continued until 1999

Mr. BURTON. Dr. Gazdar.

Mr. GAZDAR. Mr. Chairman, members of the committee, I welcome this opportunity to address you on the subject of SV–40 contamination of the polio vaccine and the role of the virus in the causation of human cancers. I've spent more than 35 years my entire professional life studying the cause of human cancers. Twenty-three of those years were spent at the National Cancer Institute.

As you have heard, several reports from laboratories around the world have demonstrated the presence of footprints of SV–40 virus in a certain select group of human tumors. You've also heard that approximately 10 percent of these reports have been negative. The virus has been associated with four types of human tumors, approximately 40 to 50 percent of these four types. These four types are brain tumors, bone tumors, mesotheliomas, and lymphomas. Three of these are very rare or relatively rare tumors; however the incidence has been increasing. Of great interest, injection of the virus into hamsters results in an identical tumor spectrum.

It defies belief that this is a coincidence that three of these rare tumors are caused by injection of the virus into hamsters and the same rare tumors in humans have also been associated with this virus. I estimate from published data that approximately 113,000 Americans will suffer from these tumors this year and 64,000 will die from their disease. Thus, approximately 50,000 tumors that occur in this country this year will contain evidence of the virus in their tumor tissues.

SV–40 is one of the most potent cancer-causing agents discovered for human cells. It's—because of—perhaps it's the most potent transforming agent, cancer-causing agent for human cells. It is widely used in laboratories, raising the spectrum that it may—its presence in human tumors is due to laboratory contamination. I was highly skeptical of the reports, and finally I decided I had the tools to investigate and, what I thought, settle the matter.

Using a technique of microsection, taking single glass slides of tumor and adjacent nonmalignant tissue, I could selectively remove the tumor cells from that glass slide as well as the nonmalignant tissues from the very same slide and analyze these independently. To my amazement, I found the virus in approximately 50 percent of human mesotheliomas and its almost complete absence in adjacent nonmalignant tissues. These experiments, in my opinion, ruled out the possibility of contamination of laboratory artifact.

I went from a skeptic to a believer. My assessment was supported by a review conducted by a panel of scientists of the National Cancer Institute chaired by Dr. Pagano and May Wong. This panel concluded that it is proven that SV–40 is present in some human tumors, and it ruled out the possibility that these were caused by laboratory artifacts. An international meeting of scientists, 80-odd scientists, held in Chicago in 2001 and chaired by two eminent scientists who never worked in this field, came to the same conclusions.

However, the presence of virus in the cancer does not prove causation because the virus may be an innocent bystander or it may be one of the causes of the tumor. To link a given agent with the cancer, one relies on both epidemiology and molecular tests demonstrating not only the presence of virus but some effect of it. The epidemiology studies, as you've heard and the Institute of Medicine has investigated, have been flawed. They're flawed because we cannot identify in these studies which subjects receive vaccination in the years under study. Also, we don't know which batches of virus were contaminated, whether the batches contained high marks of virus or low marks of virus.

For these reasons, the Institute of Medicine has declared that all epidemiology studies have been flawed and, in fact, suggest that no further epidemiology studies be performed until these deficiencies can be corrected. They did conclude that the biological evidence is strong that SV–40 is a cancer-causing virus and that the biologic evidence is of moderate strength that SV–40 exposure could lead to cancer in humans under natural conditions.

Recent molecular studies from my laboratory have convincingly demonstrated that the virus-positive tumors have different biologic properties than similar tumors that lack the virus. These studies I believe demonstrate that the virus is not just a bystander in these tumors but is having an important biologic effect, in all likelihood contributing to the causation of these tumors.

Why have we failed to make greater progress in this field? Why are we sitting here before this committee arguing whether this virus plays a role in cancer or not? It is because we have failed to make—to make progress because of a complete lack of funding, because of lack of direction from our government agencies to fund these very important issues.

Never once has the National Cancer Institute and National Institutes of Health issued a request for proposals that specifically address these issues. This lack of major funding has hampered progress and needs to be addressed. And I thank you for this opportunity.

[The prepared statement of Mr. Gazdar follows:]

Testimony of Adi F. Gazdar MD

U.S. House of Representatives
Subcommittee on Human Rights and Wellness of the Committee on Government Reform

Hearing entitled "The SV40 Virus: Has tainted polio vaccine caused an increase in Cancer?"
Washington DC, Sept. 10, 2003

Mr. Chairman and members of the Subcommittee,

I welcome the opportunity to address you on the subject of SV40 contamination of human polio vaccine and the role of the virus in the causation of human cancers.

For more than 30 years, an increasing number of reports have documented the presence of a monkey virus, simian virus 40 or SV40 in certain highly specific forms of human cancers. More than 100 reports from more than 60 laboratories world wide have documented the presence of SV40 in human tumors, although there have been a few negative studies. Approximately 40 – 50% of four types of human tumors, brain tumors, bone tumors, mesotheliomas and lymphomas contain footprints of the virus. We have recently extended these findings to human leukemias. Of great interest, injection of the virus into hamsters results in an identical tumor spectrum. These tumors will occur in an estimated 113,600 and kill 64,400 Americans this year. Thus approximately 50,000 tumors that occur in this country this year will contain evidence of SV40.

How did a monkey virus infect humans? The major source appears to be from stocks of polio vaccine, which was prepared in monkey kidney cells from the years 1955 to 1963. However, some evidence exists that the oral vaccines after this date may also have contained live SV40. Some investigators have suggested that following the administration of contaminated vaccines, SV40 may have established itself in the human population, and that the virus may be spread from human to human. However, this hypothesis remains to be tested.

SV40 is one of the most potent cancer causing agents discovered for human cells. It contains genes that take control of the growth mechanisms of infected cells, converting normal cells to tumor cells. The virus is widely used as a laboratory tool, raising the possibility of artificial contamination. Initially I was highly skeptical about the accuracy of the reports, and decided to investigate the matter personally. Using a technique known as microdissection I was amazed to find the presence of the virus in the tumor cells but not in the adjacent non malignant tissues. These experiments ruled out the possibility of contamination or laboratory artifact. I went from a skeptic to a believer. My assessment was supported by a review conducted by a panel of scientists at the National Cancer Institute and chaired by Dr. J. Pagano and M. Wong. This panel concluded that it had been definitively proven that SV40 was present in some human tumors. In agreement with my assessment, the same panel ruled out the possibility that SV40 detection was caused by laboratory artifacts.

However, the presence of a virus in a given cancer does not prove causation because the virus might simply be an innocent bystander, or be the cause or one of the causes of the tumor. To link a given carcinogen to the overall increase of a type of cancer, scientists use epidemiological analyses. To link a carcinogen to a specific cancer in a specific patient, scientists use molecular pathology. These two medical sciences are complementary: epidemiology allows us to identify the overall impact of a carcinogen in the human population, while molecular pathology studies the effect of a carcinogen in a specific patient or tumor.

The Institute of Medicine reviewed the epidemiological studies linking SV40 to human cancer and concluded that the epidemiologic studies are flawed. This conclusion was based on the observation that we are currently unable to clearly identify in which years individuals received the vaccine, who received contaminated stocks and who did not. In addition, among those who received contaminated vaccines some received stocks with low amounts of SV40 while others received stocks with high amounts of SV40 and we cannot identify these cohorts. Finally a highly reliable blood test for evidence for SV40 exposure has not been identified which further complicates epidemiologic studies.

Because the epidemiologic evidence is unsatisfactory, the evidence is inadequate to either accept or reject a causal relationship.

The biological evidence is strong that SV40 is a cancer causing virus

The biological evidence is of moderate strength that SV40 exposure could lead to cancer in humans under natural conditions.

Similar conclusions were reached by an international panel of experts who met in Chicago in 2001.

Recent molecular pathology studies from my laboratory and others has conclusively demonstrated that SV40 associated tumors have different properties than similar tumors that lack the virus. Moreover, it has been recently proven that SV40 causes specific molecular alterations in human mesothelial cells and mesotheliomas. These findings conclusively identify SV40 as a pathogen, not a passenger when present in mesothelioma. These studies provide very strong evidence that SV40 contributes towards cancer causation in some patients.

Why have we failed to make greater progress in this field? The major reason is lack of funding. Governmental agencies have failed to target this issue and have provided only token funds to a handful of scientists. Even some of the worlds experts on SV40 have failed to obtain government funding to study this issue. A major effort is needed both on the part of scientists and of the funding agencies to investigate an important health issue that may affect many thousands of Americans every year.

Mr. BURTON. You say there's no funding done to followup and to really study this issue?

Mr. GAZDAR. There's been no targeted funding. There's been a very minimal amount of funding to a handful of investigators.

Mr. BURTON. That raises the issue of whether or not the pharmaceutical companies, Lederle that produced these vaccines that may have caused these cancers, doesn't want that explored because of the possible liability that might ensue from lawsuits. And I think Mr. Kops is probably familiar with that since you were involved in litigation.

What paper were you talking about there?

There was a study, a paper on the absence of Simian Virus 40 in human brain tumors from northern India and that paper states, "Our results do not support a role for SV–40 in human brain tumors in northern India." And as I understand it, several of the people that supported that study, five of the co-authors of that paper have disassociated themselves from that. And is that correct and why would they do that?

Mr. GAZDAR. I believe you are perhaps talking about the study that Dr. Goedert talked about, the multi-lab study. Those—that——

Mr. BURTON. That's not the India study?

Mr. GAZDAR. No.

Mr. BURTON. Is this the one that Dr. Simpson was involved in?

Mr. GAZDAR. Strickler.

Mr. BURTON. Strickler.

Mr. GAZDAR. He's been involved—I'm not sure he was involved in the Indian study. Dr. Engels, who was here, was the lead author on the Indian study. He can address that issue. But it's the multi-laboratory study that Dr. Goedert spoke about which has been attacked as flawed—being highly flawed in both public and in writing, and several members of that nine-lab panel have withdrawn their association because they felt——

Mr. BURTON. Of the nine people, five have withdrawn their names as I understand it.

Mr. GAZDAR. I'm not sure of the exact number.

Mr. BURTON. Dr. Lednicky, Butel, Gisani, Jones, and Gibbs. Does that happen very often?

Mr. GAZDAR. Not to my knowledge.

Mr. BURTON. It's highly unusual, isn't it?

Mr. GAZDAR. That study took several years to get written up and published, partly because the members of that committee could not agree on the study design, how it was carried out, on the interpretation, etc.

Mr. BURTON. But you believed after, Doctor, and you say you were very skeptical at the outset on whether or not this SV–40 virus was a possible cause of tumors and cancers in people. Your attitude has changed dramatically since you actually did all this study yourself?

Mr. GAZDAR. That's right. In fact I call myself a skeptic, but frankly I simply could not believe that a monkey was suddenly turning up these rare human tumors.

Mr. BURTON. But now you believe that it can?

Mr. GAZDAR. I am firmly convinced that it not only is that but it's playing a role in the causation of tumors.

Mr. BURTON. It's causing tumors?

Mr. GAZDAR. Yes.

Mr. BURTON. What do you think we ought to do as a Congress to deal with this problem if our health agencies continue to stonewall and say we've had all kinds of tests and nothing shows up and eminent scientists have said no and there's just nothing to it? What would you suggest we do?

Mr. GAZDAR. I feel you have a part to persuade our government agencies to take a more proactive role in this issue and certainly to supply targeted funding to settle the issues. Three different committees, one convened by the NIH, by the Institute of Medicine, and this international meeting I mentioned in Chicago, have all recommended greatly increased funding to settle not only these issues but to develop new methodologies so some of our deficiencies can be corrected.

Mr. BURTON. Do we have copies of those?

We'll take a look at those and we'll write a letter to our health leaders urging them to follow that and to do that funding. But I will tell you I am convinced that our pharmaceutical companies have undue influence over our health agencies because of the liability exposure, and you can bet your bottom dollar that there will be every reason thrown up against us to try to stop us from getting to the bottom of this. Because we've had other cases—Ms. Watson and I have had cases involving mercury in vaccines, and the amount of opposition that's thrown up because of the possibility of lawsuits is just phenomenal. But what I'd like to do is have from you any recommendations that you can make so that we can submit those to HHS, FDA, and CDC to try to get them to fund that, and we'll try to keep the pressure on them to make sure that happens.

Mr. GAZDAR. I'll be glad to do that, Mr. Chairman.

Mr. BURTON. Mr. Kops, you had a lawsuit that evidently did not prevail. Can you tell us a little bit about that and what happened?

Mr. KOPS. Yes. That is a lawsuit involving a young boy who died at the age of 2. Dr. Gazdar testified in that lawsuit unequivocally that the child died from SV–40. The court had a hearing to determine whether or not there was evidence, sufficient evidence given by Dr. John Lednicky, one of the world famous scientists who is one of the scientists that the chairman has quoted from, testify that he too was under the medical certainty that this boy died from SV–40. The problem was could we prove that the given dose that this child received from an individual fill was SV–40 contaminated? We proved that the monovalent harvest were positive, positive for an adventitious agent. When the drug company reported it to the government, they said, We know what that adventitious agent is, it's a phony virus, not SV–40. Of course, they forgot to produce nine other tests which proved it couldn't possibly have been a phony agent. But the judge, hearing the arguments made by the lawyers for the drug company claiming that Dr. Lednicky's opinion was faulty because he did not do a test on the same trivalent product, therefore he would not accept his testimony.

I believe the judge was wrong. The method that this doctor used, world famous, was the identical method that the drug company uses to determine the presence or absence of SV–40. Also, the court failed to take into consideration the fact that other monovalent pools failed for specifically SV–40 and were released. The test results show it there, and the product goes out the door.

Mr. BURTON. Can we get a summary of that case from you with the relevant aspects of it so that we can take a hard look at that as well?

Mr. KOPS. Yes, I will be happy to do so.

Mr. BURTON. Ms. Grabinski, I think your testimony was sufficient, so we won't ask you any questions.

And Ms. Fisher, you and I will talk privately later because you know we work on this.

Ms. Watson.

Ms. WATSON. I just want to associate myself with something that the Chair said. I'm sitting here right now and I have a ring on, supposedly gold, and I'm having a reaction in my mouth because I have mercury amalgams, Mr. Chair, in my mouth and I'm going through the process of having them removed. It's quite a long process. I have to go out of the country to have it done, and I've already made two visits. I have four more to go.

The reason why I mentioned that is because mercury in your system, I don't care what the ADA says, is a contaminant and places those who have it at tremendous risk. I am intent on getting back to the bottom of this thing, and I do have a piece of legislation that the Chair has so kindly co-sponsored with me, and we expect to be successful.

I want to continue to take a look at those kinds of toxic materials, fluids, substances, particles or whatever that we put into the human body. Now, the question was raised do we consider ourselves to be animals? Well, biologically, physiologically, there's an answer to that. We test on animals and apply those tests to humans. So I am absolutely 100 percent committed to further research because I do think there is a connection, Mrs.——

Ms. GRABINSKI. Grabinski.

Ms. WATSON [continuing]. Grabinski, to your son's current condition and something that went into his system. I see more and more of that. My background is as a school psychologist. I had to test youth, and I can tell you we keep a record of inoculations. We keep a record of those who are in special education. I tested them to establish an IQ, make recommendations. So I'm a continuing researcher. I mean I've been in politics, took a different direction, but I'm hoping to continue that as we struggle to find the truth.

And so my question to you, Mr. Kops, is as you represent the parents and the victims, have you been able to establish legally a course of action that we can take? And I have had various industries in front of my committee when I was in the Senate because we found that silicone in breast transplants indeed were harmful to many women's health. We found also, and it was in the early 1980's, that the testing on breast cancer was done on men. How ridiculous. And so there's a continuing evolution that I mentioned. And so we had to go to court and we put companies out of business because the jury found on behalf of the victims. And we had to—

we took case law and then we made it into legislative policies, and I want you to know from the cases you've had—I want to know from the cases you've had where do you see us going with this.

Mr. KOPS. Well, I've had two different types of cases, one where the individual received the polio vaccine themselves and became paralyzed, and where their parents changed a child's diaper and became paralyzed. Those cases ended up in the Berkovitz and Sabin cases where the court held that the regulator did not enforce the regulations and the vaccine manufacturer, the same one, did not comply with the regulations.

As to the cancer issue, the problem is that no one has gone back and looked at the records. I have said in a published peer-reviewed article that appeared in the year 2000 that there are no test records. Dr. Engel was at a conference or a hearing at the IOM and he asked me a question. I was one of the people who were allowed to present a power point. He said, "Do you mean to say that all the epidemiological studies that we have conducted up to now are flawed?" I said, "Absolutely. Just go back and look at the records. You will see positive proof that SV–40 was not removed from the seeds, was not removed from the product, and released product contained the vacuolating agent SV–40." I offered to send Dr. Engels this material after the hearing.

I can tell you as of this day I have not received a request from Dr. Engels for that material, but it's now before this committee, some of it.

Mr. BURTON. You know, you hate to point fingers at any individuals because government service is a real high calling as far as I'm concerned, and most times they're not paid enough and they work long hours and they do a lot of work that the people on the street don't know about. But, you know, when our health agencies stonewall Members of Congress and keep us from getting information, it sure raises a lot of questions.

You know, this Dr. Strickler, he—one of the favored labs that he uses for the tests that he does is funded in large part and does a lot of work with Merck, Pfizer, and Wyeth, and while that doesn't apparently look like a conflict of interest, it certainly does raise some questions.

So, you know, I don't know that we can conclude a lot more from this hearing today, but what I'd like to do is have our staff contact you and get as much information as possible and we will followup on this and we will have more hearings on this, I promise you, and we will try to get from our health agencies information that they say does not exist or is hidden in the archives someplace. And we will be prepared to, if necessary, issue subpoenas to get that information.

Mr. KOPS. Thank you very much, sir.

Mr. BURTON. Do you have any final comments before we adjourn? Any additional information that you have, be sure to get that to us.

Mr. KOPS. I have submitted a written document which contains much more information and I would ask that it would become part of the record.

Mr. BURTON. Without objection, so ordered.

And we will take a hard look at this and probably get back to all of you before long.

Mr. KOPS. Thank you very much.

Mr. BURTON. Thank you very much. We stand adjourned.

[Whereupon, at 4:01 p.m., the subcommittee was adjourned.]

[Additional information submitted for the hearing record follows:]

PUBLIC HEALTH SERVICE REGULATIONS
BIOLOGICAL PRODUCTS
TITLE 42 – PART 73
AND
EXCERPTS FROM THE
PUBLIC HEALTH SERVICE ACT

Public Health Service
Publication No. 437
February 10, 1962 Revision

1798

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service

Division of Biologics Standards of the National Institutes of Health

presents evidence to demonstrate that such modification will provide equal or greater assurances of the safety, purity and potency of the vaccine as the assurances provided by such standards, and the Surgeon General so finds and makes such finding a matter of official record.

(Sec. 215, 58 Stat. 690, as amended: 42 U.S.C. 216. Interprets or applies sec. 351, 58 Stat. 702; 42 U.S.C. 262)

ADDITIONAL STANDARDS: POLIOVIRUS VACCINE, LIVE, ORAL

§ 73.110 [1] The product.

(a) *Proper name and definition.* For the purpose of section 351(a)(2) of the Act and § 73:1(k), the proper name of this product shall be "Poliovirus Vaccine, Live, Oral", followed by a designation of the form in which the vaccine is distributed by the manufacturer. The vaccine shall be a preparation of one or more live, attenuated polioviruses grown in monkey kidney cell cultures, prepared in a form suitable for oral administration.

(b) *Criteria for acceptable strains and acceptable seed virus.* (1) Strains of attenuated poliovirus Types 1, 2, and 3 used in the manufacture of the vaccine shall be identified by: (i) Historical records including origin and techniques of attenuation, (ii) antigenic properties, (iii) neurovirulence for monkeys, (iv) pathogenicity for other animals and tissue cultures of various cell types, and (v) established virus markers including rct/40, d, MS.

(2) Poliovirus strains shall not be used in the manufacture of Poliovirus Vaccine, Live, Oral, unless, (i) data are submitted to the Surgeon General which establish that each such strain is free of harmful effect upon administration in the recommended dosage to at least 100,000 people susceptible to poliomyelitis, under circumstances where adequate epidemiological surveillance of neurological illness has been maintained, and, (ii) each such strain produces a vaccine meeting the safety and potency requirements of §§ 73.114(b), 73.115, and 73.117. Susceptibility shall be demonstrated by blood tests, stool examinations and other appropriate methods.

(3) Each seed virus used in manufacture shall be demonstrated to be free of extraneous microbial agents.

(4) No seed virus shall be used for the manufacture of poliovirus vaccine unless its neurovirulence in Macaca monkeys is no greater than that of the NIH Reference Attenuated Poliovirus. The neurovirulence of the seed virus shall be demonstrated by the following tests to be performed by the manufacturer: (i) The test prescribed in § 73.114(b)(1) using seed virus as test material in place of monovalent virus pool material and (ii) the following comparative intramuscular neurovirulence test: Each of at least ten monkeys shall be injected with a total of 5.0 ml. of the seed virus under test in one or more proximate locations of either a gluteus or gastrocnemius muscle. Similar injections shall be made in another group of ten monkeys using the NIH Reference Attenuated Poliovirus. Each monkey shall be injected intramuscularly with no less than $10^{7.7}$ TCID$_{50}$ of viral inoculum. All monkeys shall be observed for 17 to 21 days and a comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the NIH Reference Attenuated Poliovirus, as prescribed in § 73.114(b)(1)(iii).

(5) Subsequent and identical neurovirulence tests shall be performed in monkeys whenever there is evidence of a change in the neurovirulence of the production virus and upon introduction

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[1] Sec. 73.110 through 73.115 amended and Sections 73.116 through 73.118 added March 25, 1961 to become effective April 24, 1961, 25 FR 2565.

of a new production seed lot and as often as necessary otherwise to establish to the satisfaction of the Surgeon General that the seed virus strains for vaccine manufacture have maintained their neurovirulence properties as set forth in § 73.114(b) (1).

(6) The Surgeon General may, from time to time, prohibit the use of a specified strain whenever he finds it is practicable to use another strain of the same type which is potentially less pathogenic for man, and that it will produce a vaccine of equivalent safety and potency.

§ 73.111 NIH reference strains.

The following NIH reference viruses shall be obtained from the Division of Biologics Standards.

NIH Reference Poliovirus, Live Attenuated, Type 1, as a control for correlation of virus titers in tissue cultures.

NIH Reference Poliovirus, Live Attenuated, Type 2, as a control for correlation of virus titers in tissue cultures.

NIH Reference Poliovirus, Live Attenuated, Type 3, as a control for correlation of virus titers in tissue cultures.

NIH Reference Attenuated Poliovirus, Type 1, as a control for correlation of monkey neurovirulence tests.

§ 73.112 Animal conditioning, personnel, and facilities.

(a) *Monkey conditioning, housing and handling.* (1) Only Macaca monkeys, or a species found by the Director, Division of Biologics Standards, to be equally suitable, in overt good health, that have reacted negatively to tuberculin at the start of the prescribed quarantine period, shall be used as the source of kidney tissue for the manufacture of poliovirus vaccine.

(2) Monkeys that have been used previously for experimental purposes shall not be used as a source of kidney tissue in the manufacture of vaccine.

(3) Monkeys to be used as a source of kidney tissue in vaccine manufacture shall be maintained in quarantine for at least six weeks prior to use in cages closed on all sides with solid materials except the front, which shall be screened. Not more than two monkeys shall be housed in one cage, and cage mates shall not be interchanged.

(4) Excluding deaths from accidents or causes not due to infectious diseases, if the death rate of any group of monkeys being conditioned in accordance with subparagraph (3) of this paragraph exceeds five percent per month, the remaining monkeys may be used for poliovirus vaccine only if they survive a new quarantine period.

(5) Each animal at necropsy shall be examined under the direction of a qualified pathologist, physician, or veterinarian having experience with diseases of monkeys, for the presence of signs or symptoms of ill health, particularly for (i) evidence of tuberculosis, (ii) presence of herpes-like lesions, including eruptions or plaques on or around the lips, in the buccal cavity or on the gums and (iii) signs of conjunctivitis. If there are any such signs or other significant gross pathological lesions, the kidneys shall not be used in the manufacture of vaccine.

(b) *Personnel.* All possible steps shall be taken to insure that personnel involved in processing the vaccine are immune to poliovirus in order to minimize the possibility that they may become excretors of poliovirus.

(c) *Facilities.* The space set aside for work with live poliovirus vaccine shall not be used for any other purpose during the vaccine processing period. All areas used for live poliovirus vaccine processing shall be decontaminated prior to the initiation of such processing. Test procedures which potentially involve the presence of micro-organisms including viruses other than the vaccine strains, or the use of tissue culture cell lines other than primary cultures, shall not be conducted in live poliovirus vaccine manufacturing areas.

§ 73.113 Manufacture of poliovirus vaccine, live, oral.

(a) *Primary cell cultures.* Only primary monkey kidney tissue cultures may be used in the manufacture of poliovirus vaccine. Continuous line cells shall not be introduced or propagated in vaccine manufacturing areas.

(b) *Virus passages.* Virus in the final product shall represent no more than five tissue culture passages from the original strain, each of which shall have met the criteria of acceptability prescribed in § 73.110(b).

(c) *Identification of processed kidneys.* The kidneys from each monkey shall be processed and the viral fluid resulting therefrom shall be identified as a separate monovalent harvest and kept separately from other monovalent harvests until all samples for the tests prescribed in the following paragraph relating to that pair of kidneys shall have been withdrawn from the harvest.

(d) *Monkey kidney tissue production vessels prior to virus inoculation.* Prior to inoculation with the seed virus, the tissue culture growth in vessels representing each pair of kidneys shall be examined microscopically for evidence of cell degeneration at least three days after complete formation of the tissue sheet. If such evidence is observed, the tissue from that pair of kidneys shall not be used for poliovirus vaccine manufacture. To test the tissue found free of cell degeneration for further evidence of freedom from demonstrable viable microbial agents, the fluid shall be removed from the cell cultures immediately prior to virus inoculation and tested in each of four culture systems; (1) Macaca monkey kidney cells, (2) Cercopithecus monkey kidney cells, (3) primary rabbit kidney cells, and (4) human cells (from one of the systems described in § 73.114(a)(6)), in the following manner: Aliquots of fluid from each vessel shall be pooled and at least ten ml. of the pool inoculated into each system, with ratios of inoculum to medium being 1:1 to 1:3 and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum. The cultures shall be observed for at least 14 days. If these tests indicate the presence in the tissue culture preparation of any viable microbial agent the tissue cultures so implicated shall not be used for poliovirus vaccine manufacture.

(e) *Control vessels.* Before inoculation with seed virus, sufficient tissue culture vessels to represent at least 25 percent of the cell suspension from each pair of kidneys shall be set aside as controls. The control vessels shall be examined microscopically for cell degeneration for an additional 14 days. The cell fluids from such control vessels shall be tested, both at the time of virus harvest and at the end of the additional observation period, by the same method prescribed for testing of fluids in the preceding paragraph (d) of this section. In addition the cell sheet in each control vessel shall be examined for presence of hemadsorption viruses by the addition of guinea pig red blood cells.

(f) *Virus harvest; interpretation of test results.* At least 80 percent of the control vessels shall successfully complete the additional 14-day observation period without microscopic evidence of cell degeneration of the tissue sheets. If less than 80 percent of the control vessels fail to complete satisfactorily the observation period, no tissue from the kidneys implicated shall be used for poliovirus vaccine manufacture. If the test results of the control vessels indicate the presence of any extraneous agent at the time of virus harvest, the entire virus harvest from that tissue culture preparation shall not be used for poliovirus vaccine manufacture. If any of the tests or observations described in paragraph (d) or (e) of this section demonstate the presence in the tissue culture preparation of any microbial agent known to be capable of producing human disease, the virus grown in such tissue culture preparation shall not be used for poliovirus vaccine manufacture.

(g) *Kidney tissue production vessels after virus inoculation—temperature.* After virus inoculation, production vessels shall be maintained at a temperature not to exceed 35.0° C. during the course of virus propagation.

(h) *Kidney tissue virus harvests.* Virus harvested from vessels containing the kidney tissue from one monkey may constitute a monovalent virus pool and be tested separately, or viral harvests from more than one pair of kidneys may be combined, identified and tested as a monavalent pool. Each pool shall be mixed thoroughly and samples withdrawn for testing as prescribed in § 73.114(a). The samples shall be withdrawn immediately after harvesting and prior to further processing, except that samples of test materials frozen immediately after harvesting and maintained at —60° C. or below, may be tested upon thawing, provided no more than one freeze-thaw cycle is employed.

(i) *Filtration.* After harvesting and removal of samples for the safety tests described in § 73.114(a), the pool shall be passed through sterile filters having a sufficiently small porosity to assure bacteriologically sterile filtrates.

§ 73.114 Test for safety.

(a) *Tests prior to filtration.* Monovalent virus pools shall contain no demonstrable viable microbial agent other than the attenuated live polioviruses intended. The vaccine shall be tested for the absence of adventitious and other infectious agents including polioviruses of other types or strains, simian agents, Mycobacterium tuberculosis, pox viruses, lymphocytic choriomeningitis virus, Echo viruses, Coxsackie viruses, and B-virus. Testing of each monovalent pool shall include the following procedures:

(1) *Inoculation of rabbits.* A minimum of 100 ml. of each monovalent virus pool shall be tested by inoculation into at least ten healthy rabbits, each weighing 1500–2500 grams. Each rabbit shall be injected intradermally in multiple sites, with a total of 1.0 ml. and subcutaneously with 9.0 ml., of the viral pool, and the animals observed for at least three weeks. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool may be used for poliovirus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of B-virus or any other viral infection.

(2) *Inoculation of adult mice.* Each of at least 20 adult mice each weighing 15–20 grams shall be inoculated intraperitoneally with 0.5 ml. and intracerebrally with 0.03 ml. of each monovalent virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least 5 additional mice which shall be observed for 21 days. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of lymphocytic choriomeningitis virus or other viral infection.

(3) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old, shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the monovalent virus pool to be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness shall be necropsied and all areas examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by the intracerebral and intraperitoneal routes and daily observed for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14 day test. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of Coxsackie or other viral infection.

(4) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighing 350–450 grams, shall be inoculated intracerebrally with 0.1 ml. and intraperitoneally with 5.0 ml. of the monovalent virus pool to be tested. The animals shall be observed for at least 42 days and daily rectal temperatures recorded for the last three weeks of the test. Each animal that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied. The tissues shall be examined both microscopically and culturally for evidence of tubercle bacilli, and by passage of tissue suspensions into at least three other guinea pigs by the intracerebral and intraperitoneal routes of inoculation for evidence of viral infection. If clinical symptoms suggest infection with lymphocytic choriomeningitis virus, serological tests shall be performed on blood samples of the test guinea pigs to confirm the clinical observations. Animals that die or are sacrified during the first three weeks after inoculation with poliovirus shall be examined for infection with lymphocytic choriomeningitis virus. Animals that die in the final three weeks shall be examined both microscopically and culturally for M. Tuberculosis. The monovalent virus pool is satisfactory for poliovirus vaccine only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with M. Tuberculosis, or any viral infection.

(5) *Inoculation of monkey kidney tissue cultures.* At least 500 doses or 50 ml., whichever represents a greater volume of virus, of each undiluted monovalent virus pool or in equal proportions from individual harvests or sub-pools, shall be tested for simian viruses in Macaca, and the same volume in Cercopithecus, monkey kidney tissue culture preparations, in a ratio of inoculum to medium of from 1:1 to 1:3, and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum, after neutralization of the poliovirus by high titer type specific nonsimian antisera. The immunizing antigens used for the preparation of antisera shall be grown in a human tissue culture cell line. The cultures shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all the tissue cultures fail to show evidence of the presence of simian viruses.

(6) *Inoculation of human cell cultures.* At least 500 doses or 50 ml., whichever represents a greater volume of virus, taken from either a single monovalent pool or in equal proportions from individual harvests or sub-pools, shall be tested in a ratio of inoculum to medium of 1:1 to 1:3, and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum, for the presence of measles virus in either (i) primary human amnion cells, (ii) primary human kidney cells, or (iii) any other cell system of comparable susceptibility to unmodified measles virus. The test material shall be neutralized with poliovirus antiserum of non-simian derivation if the tissue culture cell system used is susceptible to poliovirus. The culture shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of measles virus.

§ 73.115 Potency test.

The concentration of live virus expressed as $TCID_{50}$ of each type in the vaccine shall constitute the measure of its potency. The accuracy of the titration to determine the concentration of live virus in the lot under test shall be confirmed by performing a titration with the NIH Reference Poliovirus, Live, Attenuated of the appropriate type as a check on titration technique. The concentration of each type of live virus contained in the vaccine of the lot under test shall be between 200,000 and 500,000 $TCID_{50}$ per human dose.

§ 73.116 General requirements.

(a) *Repeat tests.* Tests may be repeated when it is demonstrated that the results were due to faulty test techniques.

(b) *Final container tests.* Tests shall be made on final containers for identity and safety in accordance with § 73.72. The final container sterility test need not be performed provided aseptic techniques are used in the filling process.

(c) *Consistency of manufacture.* No lot of vaccine shall be released unless each monovalent pool contained therein is one of a series of five consecutive pools of the same type, each pool having been manufactured by the same procedures, and each having met the criteria of neurovirulence for monkeys prescribed in § 73.114(b)(1), and of in-vitro markers as prescribed in § 73.114(b)(3).

(d) *Dose.* The individual human dose of vaccine shall contain from 200,000 to 500,000 $TCID_{50}$ of each type of virus that is in the final product

(e) *Labeling.* Labeling shall comply with the requirements of §§ 73.50 to 73.55, inclusive. In addition the label or a package enclosure shall include the identification and source of the virus or viruses contained in the vaccine, the tissue medium on which the virus or viruses were propagated, stabilizers and preservatives if any, and the type and calculated maximum amount of antibiotics.

(f) *Dating.* (1) The expiration date in no event shall be more than two years after the date of manufacture as defined in § 73.82(a) provided the product is maintained in the frozen state, (2) the expiration date shall be no more than one year from the date of issue provided that the product is maintained in the frozen state, and (3) the expiration date shall be no more than seven days from the date of issue if issued as a liquid and provided it is maintained at a temperature no higher than 10° C.

(g) *Samples and reports.* For each lot of vaccine, the following materials shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda 14, Maryland:

(7) *Inoculation of rabbit kidney tissue cultures.* At least 500 ml. of virus pool taken from either a single monovalent pool or in equal proportions from individual harvests or sub-pools, shall be tested in a ratio of inoculum to medium of from 1:1 to 1:3, and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum, in primary rabbit kidney tissue culture preparations for evidence of B-virus. The culture shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of B-virus.

(b) *Tests after filtration.* The following tests relating to safety shall be performed after the filtration process, on each monovalent virus pool or on each multiple thereof (monovalent lot):

(1) *Neurovirulence in monkeys.* Each monovalent virus pool or monovalent lot shall be tested in comparison with the NIH Reference Attenuated Poliovirus for neurovirulence in Macaca monkeys by both the intrathalamic and intraspinal routes of injection. A preinjection serum sample obtained from each monkey must be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 $TCID_{50}$ of each of the three types of poliovirus. The neurovirulence tests are not valid unless the sample contains at least $10^{7.0}$ $TCID_{50}$ per ml. when titrated in comparison with the NIH Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days, under the supervision of a qualified pathologist, physician or veterinarian, and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(i) *Intrathalamic inoculation.* Each of at least ten Macaca monkeys shall be injected intrathalamically with 1.0 ml. of virus pool material containing at least $10^{7.0}$ $TCID_{50}$ per ml. and each of at least ten additional Macaca monkeys shall be injected intrathalamically with 1.0 ml of a 10^{-1} dilution thereof. Comparative evaluations shall be made with the virus pool under test and the NIH reference material. Only monkeys that show evidence of inoculation into the thalamus shall be considered as having been injected satisfactorily.

(ii) *Intraspinal inoculation.* Each of a group of at least five Macaca monkeys shall be injected intraspinally with 0.2 ml. of virus pool material containing at least $10^{7.0}$ $TCID_{50}$ per ml. and each monkey in four additional groups of at least five Macaca monkeys shall be injected intraspinally with 0.2 ml. of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions thereof, respectively. Comparative evaluations shall be made with the virus pool under test and the NIH reference material. Only monkeys that show microscopic evidence of inoculation into the gray matter of the lumbar cord shall be considered as having been injected satisfactorily.

(iii) *Determination of neurovirulence.* At the conclusion of the observation period comparative histopathological examinations shall be made of the lumbar cord, cervical cord, lower medulla, upper medulla and mesencaphalon of each monkey in the groups injected with virus under test and those injected with the NIH Reference Attenuated poliovirus, except that for animals dying during the test period, these examinations shall be made immediately after death. The animals shall be examined to ascertain whether the distribution and histological nature of the lesions are characteristic of poliovirus infection. A comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the NIH Reference Attenuated Poliovirus with respect to (*a*) the number of animals showing lesions characteristic of poliovirus infection, (*b*) the number of animals showing lesions other than those characteristic of poliovirus infection, (*c*) the severity of the lesions, (*d*) the degree of dissemination of the lesions, and (*e*) the rate of occurrence of paralysis not attributable to the mechanical injury resulting from inoculation trauma. The virus pool under test is satisfactory for poliovirus vaccine manufacture only if at least 80 percent of the animals in each group survive the observation period and if a comparative analysis of the test results demonstrate that the neurovirulence of the test virus pool does not exceed that of the NIH Reference Attenuated Poliovirus.

(2) *Test for virus titer.* The concentration of living virus in each monovalent virus pool or lot shall be determined, using the NIH Reference Poliovirus Live, Attenuated of the same type as a control. The test shall be a 50 percent end-point titration calculation (TCID$_{50}$), performed with either groups of ten tubes at 1 log dilution steps or groups of five tubes of 0.5 log dilution steps, or a test of demonstrated equivalent sensitivity. Acceptable titrations of the reference virus shall not vary more than ±0.5 log from its labeled titer.

(3) *Tests for In-vitro Markers.* A test shall be performed on each monovalent virus pool or each monovalent lot resulting therefrom, using the rct/40 Marker and at least one of the other marker methods described below. The test results shall demonstrate that the virus under test and the seed virus have substantially the same marker characteristics.

(i) *rct/40 Marker.* Attenuated strains which grow readily at 40° C. (±0.5° C.) are classified as rct/40 positive (+) in contrast to the rct/40 negative (—) strains which show an increased growth of at least 100,000 fold at 36° C. over that obtained at 40° C. Comparative determinations shall be made in either tube or bottle cultures.

(ii) *d Marker.* Attenuated strains which grow readily at low concentrations of bicarbonate under agar are classified as d positive (+) in contrast to the d negative (—) strains which exhibit delayed growth under the same conditions. The cultures shall be grown in a 36° C. incubator either in stoppered bottles or in plates in an environment of 5 percent CO$_2$ in air.

(iii) *MS Markers.* Attenuated strains which grow more readily on monkey stable (MS) cells are classified as MS positive (+) in contrast to the MS negative (—) strains. Comparative determinations shall be made in either tube cultures or in bottle cultures under agar.

(4) *Test for sterility.* The final bulk vaccine of each monovalent virus pool, or each monovalent lot resulting therefrom, shall be tested for sterility in both fluid Thioglycollate and Sabouraud media by the procedure prescribed in § 73.73 with a sample of no less than 10 ml. for each test.

(1) All protocols relating to the history of manufacture of each lot of vaccine, and the results of all tests performed.

(2) A one liter bulk sample of each final monovalent pool having a virus titer of no less than $10^{7.0}$ $TCID_{50}$ per milliliter, except that if the titer is greater, a correspondingly smaller volume may be submitted.

(3). A total of no less than a 200 milliliter sample of the vaccine in final labeled containers.

§ 73.117 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design, by oral administration of the product. Such clinical trials shall be conducted with five consecutive lots of poliovirus vaccine which have been manufactured by the same methods, each of which has shown satisfactory results in all prescribed tests. Type specific neutralizing antibody (from less than 1:4 before vaccine treatment, to 1:16 or greater after treatment) shall be induced in 80 percent or more of susceptibles when administered orally as a single dose or in excess of 90 percent of susceptibles when administered orally after a series of doses. A separate clinical trial shall have been conducted for each monovalent and each polyvalent vaccine for which license application is made.

§ 73.118 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Poliovirus Vaccine, Live, Oral, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Surgeon General so finds and makes such finding a matter of official record.

(Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Interpret or apply Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262)

Poliovirus Vaccine, Live, Oral

Sabin Strains Types 1, 2, and 3

BERKOVITZ V. LEDERLE

Orimune®

MEDICAL ADVISORY DEPARTMENT
LEDERLE LABORATORIES

Address Inquiries To

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LEDERLE LABORATORIES
PEARL RIVER • NEW YORK

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What is ORIMUNE®?

ORIMUNE®* 1, 2, and 3 (Poliovirus Vaccines, Live, Oral, M valent) *Lederle* are suspensions of living, attenuated poliomye viruses. Each of the 3 vaccines contains a single strain of viru the immunological type designated by the number. The three vac strains have been carefully chosen for their lack of ability to in and damage the human central nervous system. ORIMUNE is the commercially available American-made vaccine to offer protec against all 3 types of polio *by the oral route.*

Live virus vaccines, made from attenuated strains, are not an i vation to preventive medicine. Indeed, the first vaccine used in ventive medicine – the cowpox or vaccinia virus, which gave the n vaccination to protective immunization in general – is a natur occurring mild strain, closely related to the virulent smallpox vi Other attenuated viruses have been developed in the laborat beginning with the attenuated yellow fever virus introduced in 19

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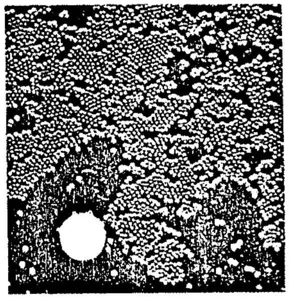
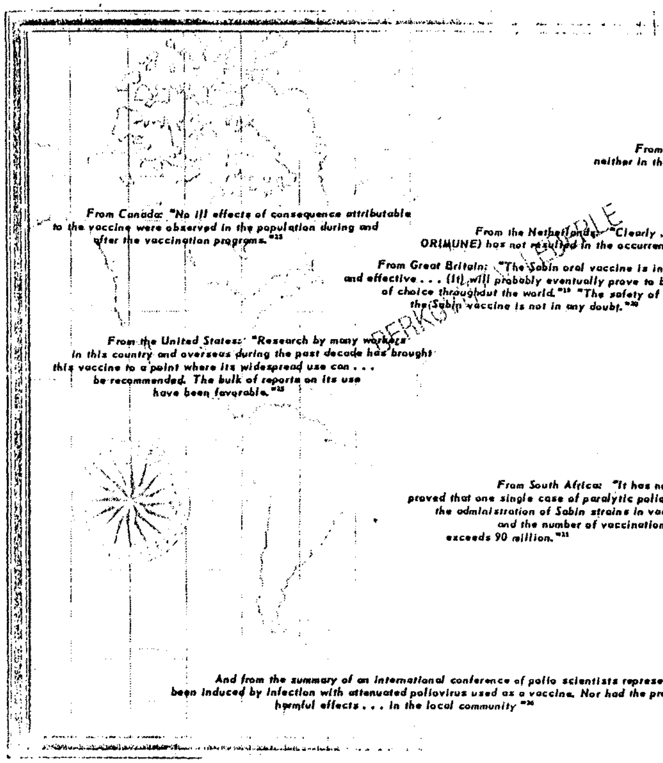


(From Schwerdt, 32) *40,000X*

Type 2 polio virus particles

*ORIMUNE® is the registered trademark of Lederle Laboratories

What Do Leading Medical Authorities Say about Live Poliovirus Vaccine?



From behind the Iron Curtain: "Live Sabin vaccine is safe — neither in the course of the vaccination nor afterwards adverse side-effects or complications were recorded."[1]

From Canada: "No ill effects of consequence attributable to the vaccine were observed in the population during and after the vaccination programs."[23]

From the Netherlands: "Clearly . . . the (use) of the vaccine (same strains as ORIMUNE) has not resulted in the occurrence of clinical poliomyelitis."[13]

From Great Britain: "The Sabin oral vaccine is in fact safe and effective . . . (It) will probably eventually prove to be the vaccine of choice throughout the world."[19] "The safety of the Sabin vaccine is not in any doubt."[20]

From the United States: "Research by many workers in this country and overseas during the past decade has brought this vaccine to a point where its widespread use can . . . be recommended. The bulk of reports on its use have been favorable."[25]

From Asia: "The attenuated type 2 poliovirus (same strain as ORIMUNE 2) was administered to 198,965 children without causing any cases of paralytic poliomyelitis."[14]

From South Africa: "It has not yet been proved that one single case of paralytic poliomyelitis has been caused by the administration of Sabin strains in vaccines or their contacts, and the number of vaccinations now probably exceeds 90 million."[31]

And from the summary of an international conference of polio scientists representing 20 nations: "Poliomyelitis had not . . . been induced by infection with attenuated poliovirus used as a vaccine. Nor had the progeny of the vaccine virus induced harmful effects . . . in the local community"[26]

Who Should Receive ORIMUNE?

The answer to this question is *emphatically*, EVERYONE.

THERE ARE NO KNOWN CONTRAINDICATIONS TO ORIMUNE.

The Surgeon General's Advisory Committee of Poliomyelitis Control has specifically recommended[30] that certain conditions "previously of concern" *not* be considered contraindicative to vaccination with live, oral polio vaccine.

> These recommendations include *tonsillectomy* and *tooth extraction; pregnancy;* and *steroid therapy.*
>
> They include *agammaglobulinemia*, although in this condition antibody response may not be complete.
>
> *Concurrent smallpox vaccination, diphtheria and tetanus toxoids and pertussis vaccine combined inoculation*, or administration of other immunizing agents is not contraindicative to the use of ORIMUNE, according to the recommendations.
>
> *Penicillin hypersensitivity* is also mentioned as *not* contraindicative to ORIMUNE.

Sensitivity to other antibiotics should not be of concern, either. Each 2 cc. dose of ORIMUNE 1, 2 or 3 contains less than 1 microgram of each of the antibiotics streptomycin, neomycin, and nystatin. These compounds are so poorly absorbed after oral administration that these small quantities should not cause undesirable reactions even in hypersensitive individuals.

Because other enteroviruses, such as the ECHO or Coxsackie viruses, may interfere with the oral vaccine and prevent or diminish the desired response, and because the efficacy of an orally administered live poliovirus vaccine depends on multiplication within the intestinal tract, it is suggested that immunization be postponed if there is persistent vomiting or diarrhea, or known infection with another enterovirus.

Previous injections of killed polio vaccines are in no way contraindicative to the administration of ORIMUNE. Also, a course of oral immunization begun with another brand of vaccine may be completed with ORIMUNE.

The clinical judgement of the responsible physician should prevail in every case.

Who Should Receive ORIMUNE First?

The United States Public Health Service has issued recommendat
regarding priorities for the use of the poliovaccines, during the p
myelitis season of 1962 when supplies may be limited.[24]

1. Vaccination programs in areas threatened with epidemics. See the specific recommendations.
2. Routine immunization of infants, beginning at 6 weeks of age and completed in one year.
3. Pre-school children who have not yet been fully immunized.
4. Older unimmunized groups, particulary young adults and the parents of young children.

For more specific information, the physician is referred to this re
This statement was published *in toto* in the *Journal of the Amer*
Medical Association on April 15, 1962.

Storage and Use of ORIMUNE

Poliovirus Vaccine, Live, Oral will retain the stated potency until
expiration date on the package (one year from release by the m
facturer) if stored in the freezer compartment of a refrigerator.
MUNE can be stored for 7 days at ordinary refrigerator temperat
(2° to 10° Centigrade or 36° to 50 F.) without loss of potency.

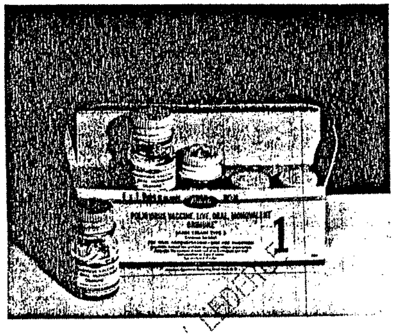
The only effect of a temperature higher than 10° Centigrade (50°
or storage at refrigerator temperatures for longer than 7 days o
freezer temperatures for longer than one year, would be a poss
loss of potency of the vaccine.

ORIMUNE contains sorbitol ($CH_2OH(CHOH)_4CH_2OH$) in a conc
tration of 45% (weight/volume) as a vehicle for the live virus.
cause of this sorbitol content, ORIMUNE will remain fluid at tem
ature above -14° C. (+7° F.). It is not necessary to maintain it
solid frozen state.

ORIMUNE is supplied in boxes of 5 single-dose vials of Type 1
or 3, and in multiple-dose bottles of each type. The packaging
labels of ORIMUNE are printed in Lederle's familiar blue and yell
Possible confusion of types is minimized by a large color-co
Arabic numeral designating the type of each package. This nun
appears on the front

s... The color codes specified by the Public Health Service for monovalent attenuated polio vaccine are:

Type 1 -- *Red*
Type 2 -- *Yellow*
Type 3 -- *Green*



(Lederle Photo)

Five single-dose vials of ORIMUNE 1

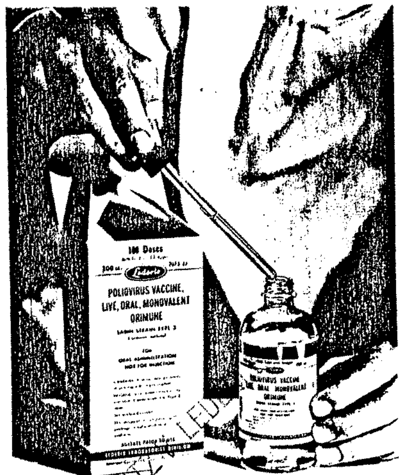
The vaccine as supplied is ready for use. The multiple-dose bottles are furnished with graduated droppers, which can be used to dispense the recommended dose of 2 cc. into a teaspoon or small cup. Infants and small children may receive ORIMUNE directly from the dropper. ORIMUNE is a clear liquid, with a slightly sweet taste.

If the multiple-dose bottles are frozen, they should be agitated after thawing to insure homogeneity of the contents.

Since the establishment of immunity requires contact of the vaccine virus with the intestinal mucosa, it may be advisable to give the vaccine at a time other than just before or after a full meal

Underdosage may occur through spillage, improper ingestior through emesis in infants. While immunity might conceivably be tablished from only a small fraction of the dose, it could onl determined by complex laboratory techniques. The remedy for su situation would be to feed another dose of ORIMUNE.

Each 2 cc. dose of ORIMUNE contains 200,000–500,000 tis culture infective doses ($TCID_{50}$) of the specified strain of viru



(Lederle Photo)

A 100-dose bottle of ORIMUNE 3

accordance with government regulation. This unit of measurem ($TCID_{50}$) is defined as the smallest quantity of a virus suspens which will, when inoculated into tissue culture under a standard of conditions, result in the infection of at least 50% of such preparatio

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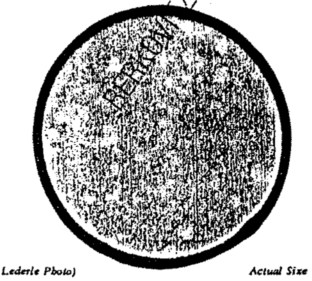
W. are Attenuated Strains?

Virus populations, like any other natural populations, may not be homogeneous and may contain a few members which differ from the rest in one way or another. By inoculating large numbers of virus particles into a medium different from that favored by the virus in nature, a few of the variants may find themselves at a reproductive advantage relative to the "wild" type. After many passages the strain becomes "adapted" to the new medium; that is, the variant viruses replace the wild type almost completely.

The introduction of the plaque-purification technique by Dulbecco[2] made further advances in attenuation possible. In this technique, virus "harvests" are greatly diluted and quantities of the fluid small enough to contain, on the average, 20 to 100 virus particles are spread out over layers of fully grown susceptible cells, and they are immobilized under a thin layer of nutrient fluid containing agar. Individual viruses will remain separate and will form colonies or plaques at some distance from each other.



(Lederle Photo) Actual Size

Virus plaques on a monolayer tissue-culture

Each plaque, representing the progeny of only one virus particle, is tested and the most avirulent plaques are selected. The homogeneity of these populations is further assured by repetition of the full cycle 3 or 4 times. By this method, virus strains can be chosen which are nonpathogenic for man, but can still induce immunity against their "wild" counterparts. These are "attenuated" strains

Why an Oral Polio Vaccine?

Oral, attenuated polio vaccine was developed primarily becau was believed that such vaccine could produce higher antibody l than inactivated vaccines. This expectation has been realized; furthermore, the proportion of susceptibles – those without circul antibodies before vaccination – who are converted to antibody-pos status after vaccination is greater with live, attenuated vaccine with the killed vaccine.[3]

In addition to this quantitative difference in the efficacy of the cine, no injection is required, so that the live vaccine is easi administer on a mass basis, and is acceptable to more people. A at least 4 injections of killed vaccine must be given, over a pe of 12 to 20 months, to achieve an acceptable level of protec whereas only 3 oral doses, one of each type, at 6 week intervals, necessary for immunization with ORIMUNE monovalent vaccines.

Oral Vaccine Confers Intestinal Immunity

Live, oral polio vaccine has another advantage, important above others, in its ability to induce resistance in the intestinal trac reinfection with poliovirus, thus simulating the effects of na infection. The poliovirus is an enterovirus, entering the body by oral route. Infection of the central nervous system is essentiall infrequent complication of a benign intestinal infection. Polioviru fection is highly contagious, but only a very small proportio naturally infected subjects – according to some estimates, less 10 per 1,000[4] – develops paralytic symptoms. Recovery from suc intestinal infection is followed by a long-lasting local *and* gen resistance to reinfection with homotypic poliovirus. The mecha of this resistance is not understood, but it has been demonstr that persons known to have had intestinal infections with polio will excrete, at the most, trace amount of homotypic virus upo exposure.[5,6] On the other hand, circulating antibodies induce killed vaccine, even when present at very high levels, cannot a prevent the establishment of an intestinal infection in the absenc a previous intestinal infection.[7,8,9]

This twofold advantage of live, oral vaccine is important to vaccinee because killed vaccine injections alone do not pro uniform protection against paralytic polio. In the United State 1961, about 1 person in every 2 (54% of the population under 60) had at least 3 injections of killed vaccine[10] but 20%

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Dosage Schedules

The Public Health Service recommends that live, oral poliovirus vaccine be given according to the following schedule.

Dose: 2 cc. ORIMUNE orally, per administration.

For Infants: *(Immunization should begin at 6 weeks to 3 months of age).*
First dose: Type 1
Second dose: Type 3, 6 weeks after first dose
Third dose: Type 2, 6 weeks after second dose
Fourth dose: Types 1, 2, and 3, 6 months or longer after the third dose. The fourth dose may be given as 2 cc. of each type simultaneously, or it may be administered as a second course at six-week intervals.

For all others: *(including community use)*
First dose: Type 1
Second dose: Type 3, 6 weeks after first dose
Third dose: Type 2, 6 weeks after second dose

While the need for booster doses in children and adults has not yet been established, booster doses might be considered every 2 to 4 years, or under the threat of an epidemic.

When ORIMUNE is given as a "booster" dose to persons who have had a *complete* course of killed vaccine injections, it should be given as three separate doses at six-week intervals, in the order Type 1, Type 3, Type 2.

Infants *under* 6 weeks of age who are fed ORIMUNE will suffer no ill effects, but at this age it is possible that maternal antibodies will prevent the establishment of immunity.

Homotypic immunity is normally developed to a high level within 4 weeks after live, oral monovalent poliovirus vaccine is given. Since some individuals will maintain intestinal infection for a somewhat longer period, an interval of 6 weeks between doses is recommended under normal circumstances to avoid possible interference between the vaccine strains. Type 2 is administered last, because it characteristically produces a longer period of intestinal infection than do the other types. Type 2 would be the logical choice for last administration in any case, since it accounts for only about 1% of the paralytic cases of poliomyelitis in the United States, the rest being about equally divided between Types 1 and 3.[11]

The phenomenon of interference is not completely understood, bu
does appear that any other intestinal virus can compete for the s
ecological niche occupied by polioviruses. Once an infection wit
enterovirus, such as ECHO or Coxsackie, is established, it is d
cult for a subsequently invading virus to dislodge it and estab
itself. Thus an attenuated poliovirus vaccine will be of no valu
the presence of an incubating wild or virulent poliovirus infection.

If vomiting develops within a few days after administration of O
MUNE, it is not due to the vaccine. Emesis is not an uncom
event, especially among pediatric patients. Such a report may sug
the concurrent existence of another enterovirus infection, wh
might have interfered with the vaccine strain administered. In
event of such a report, the physician may wish to consider rev
cination with the same strain of ORIMUNE.

The existence of viremia in any kind of poliovirus infection is v
difficult to demonstrate. Some authorities believe that viremia
necessary for the development of circulating antibodies. If this is
case, viremia must occur in nearly every susceptible vaccine
receives ORIMUNE. As has been pointed out above, approximat
one person out of every 20 in the world has been given live,
poliovirus vaccine made from these particular strains; not one
been shown to have suffered any ill effects.

Transmission of attenuated poliovirus infection from vaccines to th
contacts has been shown to occur, especially within families.[15]
average number of contacts infected by each vaccinee in this st
was about one. The Public Health Service recommends that any c
munity immunization program aim at vaccinating no less than 80%
preschool children in all socioeconomic groups. It is considered
if this goal is achieved, direct or contact infection will result in
immunization of virtually the entire community.

The Public Health Service recommends that individuals who h
received partial courses of killed, injectable polio vaccine compl
their immunization with either injected or oral polio vaccine,
cording to availability and preference, *as long as a complete cou*
of one or the other is given. Immunity against all three types of po
virus can normally be established by a full course of ORIMUNE wi
12 to 16 weeks.

ORIMUNE 1, 2, and 3 are produced and tested in accordance with the regulations of the United States Public Health Service for production of Poliovirus Vaccine, Live, Oral. The manufacturer makes no representation or warranty, express or implied, with respect to the merchantability or fitness for use of these vaccines other than that they have been produced in accordance with the standards for their production prescribed by the United States Public Health Service and applicable thereto at the time of their release by the manufacturer.

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MEDICAL COMMUNICATION DEPARTMENT
MEDICAL RESEARCH SECTION
LEDERLE LABORATORIES
APRIL
1962

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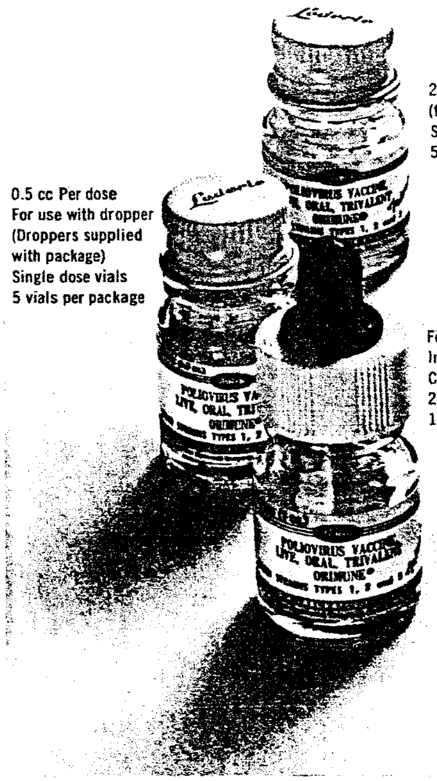

2 cc Per dose
(for general use)
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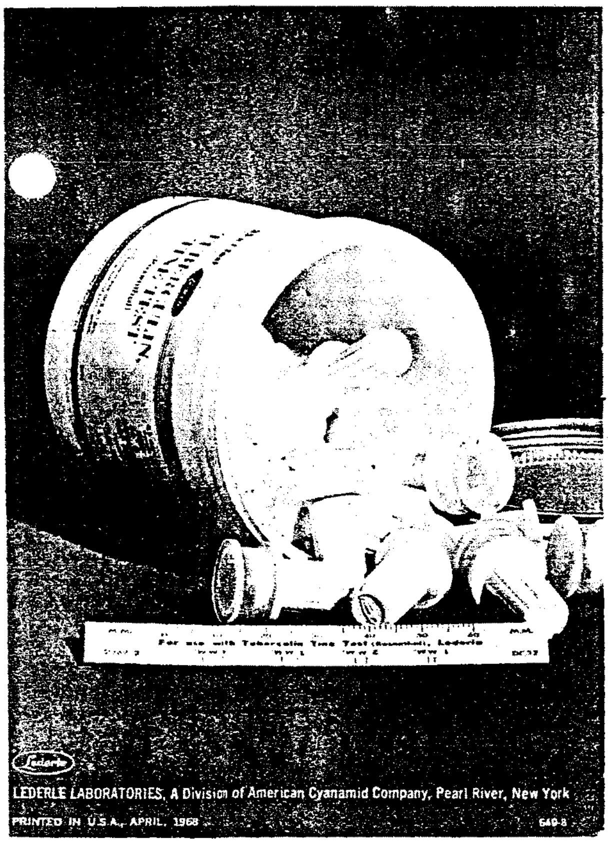

LEDERLE LABORATORIES, A Division of American Cyanamid Company, Pearl River, New York
PRINTED IN U.S.A., APRIL, 1968 649-8

at least 45 monkeys, every one of which must have been demonstrated to be completely free of poliovirus antibodies. If the vaccine passes both clinical and histopathological comparisons with the rigorous standards of the reference strains, the vaccine can then be titrated and 2 final tests for bacterial sterility made. Only if every one of these many tests has been passed, can the vaccine be diluted and the final product be labelled Poliovirus Vaccine, Live, Oral.

Additional Requirements for Manufacturing License

Every lot of live polio vaccine released for commercial distribution must pass the exhaustive test schedule described. Before Lederle could be licensed to manufacture the vaccine, however, further requirements had to be met for *each* virus type. *Five consecutive lots* had to be produced, each of which passed all of the many tests. Then these 15 lots, 5 of each type, were used in a controlled clinical trial, in accordance with government regulations. Conversion of circulating antibody status from completely negative to at least 1:16 titer had to be demonstrated in at least 80% of susceptibles.

Lederle's new 3 million dollar facility for manufacturing and testing oral polio vaccine is subject to Public Health Service inspection, and cannot be used for any other purpose during the manufacture of attenuated poliovirus vaccine. The 250 foot long sterile filling line, in which the vaccine is sealed in its final containers, is believed to be the longest in the world. No procedures involving even the potential presence of nonpoliovirus microorganisms, or tissue cultures other than the primary cell lines required, may be conducted in the vaccine production area. Personnel involved must be thoroughly immunized against poliomyelitis. They cannot wear street clothes in the laboratories, and they cannot come in contact with other infectious agents in other laboratories on the same day.

Behind the government's schedule of requirements, as extensive as it is, stands the accumulated knowledge and experience of Lederle's 14 year pioneering research in the field of live polio vaccine, in the best tradition of a 56-year history of producing biological products for the medical profession. The first live polio vaccine ever given was a Lederle vaccine[4]. Lederle has been active in live poliovirus vaccine research longer than any other commercial manufacturer in the world.

What About "Reversion?" Can the Vaccine Strain Become Virulent Again After Transmission by a Vaccinee?

In the early stages of live polio vaccine development, many scientis
voiced concern over the possibility of widely disseminated vacci
strains reverting and causing paralytic disease among contacts. T
viruses excreted after a vaccine infection sometimes differ slight
from the strains which went into the vaccine. Such "first human pa
sage" strains *may* be slightly more neurovirulent, when injected
rectly into the spinal cord of monkeys, than was the original vacci
strain.

Extensive studies have shown, however, that this slight tenden
towards increased neurovirulence in laboratory tests does *not* co
tinue *beyond* the first human passage. Viruses recovered after
many as 10 human passages showed no more neurovirulence in mo
keys than the first human passage isolation. The great majority
excreted vaccine strains show *no* increase in neurotropism at all.
It is now generally agreed among polio scientists that there is
danger of vaccine reversion to virulence after human passage.

Can Oral Polio Vaccine Cause Other Diseases?

The safety regulations discussed above include many general sa
ty tests. There are also tests designed especially to detect c
tain potentially pathogenic organisms, such as the vacuolating age
(SV-40).[24] Every production pool of ORIMUNE is carefully test
many times for SV-40, and any lot in which it is found is rejected.

Ritchey
Exhibit 2
2/27/01

Brown F, Lewis AM (eds): Simian Virus 40 (SV40): A Possible Human Polyomavirus.
Dev Biol Stand. Basel, Karger, 1998, vol 94, pp 217-219

Product Quality Control Testing for the Oral Polio Vaccine

B. Brock, L. Kellener, B. Zlotnick

Wyeth-Lederle Vaccines and Pediatrics, Pearl River, NY, U.S.A.

Key words: Sabin oral polio vaccine, quality control testing, SV40

Abstract: A brief overview regarding the quality control testing of the Sabin oral polio vaccine is provided. Product testing procedures and specifications are established through product license agreements between the vaccine manufacturer and the FDA Center for Biologics Evaluation and Research. The manufacture and testing of ORIMUNE® is a multi-stage process that is closely monitored by the FDA following explicit protocols and requires extensive quality control testing at various stages.

INTRODUCTION

The oral polio vaccine is a trivalent preparation comprising attenuated Sabin strains of polio virus types 1, 2, and 3 in an oral dosage form. The vaccine induces an immune response comparable to the natural disease and is credited with the control and eradication of wild-type polio disease in the United States.

Lederle Laboratories has distributed over 650 million doses since the licensure of ORIMUNE® in 1963. The viral content of the vaccine is specified by FDA regulations. The three individual polio virus types are combined in specific ratios to assure that all three strains immunize effectively.

MATERIALS AND METHODS

Cell culture preparation

The cell culture substrate is prepared in primary monkey kidney cells obtained from green monkeys that do not harbour SV40 virus. The monkeys used as a source of kidney tissue are purpose-bred in isolated breeding colonies. These animals are tested for tuberculosis and viral antibodies and are held in isolation quarantine under strict veterinary supervision until use. A kidney perfusion process is performed under aseptic conditions which liberates kidney (renal epithelial) cells in preparation for cell culturing. Perfused kidneys are then delivered to the cell culture laboratory. The cells are dispersed into monocellular suspensions under aseptic conditions and then diluted in a growth medium containing the nutrients necessary for growth and replication. Cells are planted into roller bottles and incubated to form a cell monolayer.

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2
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Cells are grown and observed for at least 11 days in the cell culture laboratory. After cell growth is completed, 75% of the roller bottles are sent to the virus production laboratory for polio virus inoculation. The remaining 25% of the roller bottles are sent to quality control for testing.

Cell culture testing

Fluids from all the roller bottles of a single kidney are pooled and tested to detect the presence of any transmissible, microbial agent by inoculation into four cell lines: (i) Cercopithecus monkey kidney cells (primary CMK cells) for an initial 14 days; followed by a 14-day subculture, again in CMK cells; (ii) Rhesus monkey kidney cells (primary RMK cells) for at least 14 days; (iii) primary rabbit kidney cells for at least 14 days; and (iv) BSC-1 cells (continuous green monkey kidney cells) for at least 14 days.

Twenty-five percent of all the cell culture bottles are sent to Quality Control for testing purposes. These cell culture bottles are then observed in their original control bottles for at least 14 more days, followed by a test to detect haemabsorptive viruses.

At day 4 of the Quality Control observation period, fluids are removed from the original bottles and again tested in the same four cell systems previously described to detect the presence of any transmissible microbial agent. This testing includes the additional 14-day subculture on CMK cells.

At day 14 of the Quality Control observation period, fluids are again removed from the original bottles and again tested in the same four cell systems, including a 14-day subculture in CMK cells. Therefore, every individual cell batch is observed for a total of more than 50 days in culture. The appearance of an adventitious agent at any stage of testing results in rejection of the cell batch.

Virus production

The remaining 75% of the cell culture bottles are sent to production for virus inoculation. One of the Sabin attenuated strains is prepared to inoculate the production bottles. Master polio virus seed stocks are maintained in a viable state in liquid nitrogen storage.

Master viral strains have been prepared in the presence of SV40 virus neutralizing antiserum. All subsequent working seed strains have been prepared in CMK cells and screened to assure they are free of SV40 virus.

The same level of virus is used for each group of bottles inoculated. Production bottles are examined and records checked. Only one polio virus type is processed at a time. At the appropriate time following polio virus infection, fluids from infected tissues which contain polio virus are harvested.

Viral harvest testing

Viral harvest samples are sent to the Quality Control laboratory for evaluation and the rest of the harvested fluids are stored frozen until testing is completed. Fluids collected from these bottles are tested to detect the presence of any transmissible microbial agent in CMK cells for 14 days, followed by a subculture in CMK cells for a further 14 days. Viral harvest fluids are also tested in Rhesus monkey kidney cells, rabbit kidney cells, and BSC-1 cells, all for 14 days. Additionally, samples are tested to demonstrate the absence of mycoplasma.

Quality Assurance releases a virus harvest for further processing when all testing has been completed with satisfactory results for (i) the original cell culture, (ii) the cell culture fluids and subcultures, and (iii) the viral harvest samples.

Monopool testing

When the appropriate number of harvests for a single polio virus type are released by Quality Assurance, they are thawed and combined to form a monopool. Samples from an unfiltered, prorata monopool are tested to ensure freedom from adventitious agents in rabbits, guinea pigs, adult mice, and newborn mice. The production monopool is then passed through a 0.22 micron filter. Samples are taken for monopool testing by Quality Control to include testing for potency, testing for polio neurovirulence, and testing for markers of attenuation. The appearance of any adventitious agent at any stage of testing results in rejection of the monopool. This process is repeated for each monopool virus type.

Monopool release

A document is prepared containing the production history and test results on the monopool by Quality Assurance. This document is submitted to the FDA Center for Biologics Evaluation and

Research, along with monopool samples for testing. The FDA reviews the manufacturer's test results, performs tests as appropriate, and provides notification of the release of the monopool for further manufacture.

Trivalent vaccine release

Released monopools, of each type, are combined with diluent to make a trivalent vaccine bulk preparation. Samples are tested by Quality Control for potency and sterility. The vaccine is aseptically filled into single dose final containers. Samples are tested by Quality Control for potency, identity, and safety. Final container samples are also sent to the FDA with a final protocol for the release of the final filled container vaccine for distribution.

Concluding Remarks

Extensive quality control testing of the Sabin oral polio vaccine is performed throughout the multi-stage manufacturing process. Testing of the cell culture and viral harvest preparations to assure the absence of adventitious agents uses large cell production volumes (25% of total), multiple detection systems, repetitive testing, and lengthy observation periods (cumulatively beyond 50 days). Over 4,000 individual cell culture observations are made during the quality control testing of a single trivalent bulk lot. Any product contamination with an adventitious agent observed at any point results in rejection of the materials for product use.

Monopool and trivalent vaccine testing is performed to assure the safety and potency of the product. This testing includes well-established and sensitive animal tests in newborn mice, adult mice, guinea pigs, rabbits and rhesus monkeys, as well as in-vitro laboratory assays. Samples from every product monopool and final trivalent vaccine lot are provided to the FDA government laboratory for concurrent testing. The FDA Center for Biologics Evaluation and Research reviews the testing and manufacturing protocol and provides component or product release to the manufacturer for every individual product monopool and final trivalent vaccine lot before commercial distribution for use in humans.

Reference

1 Polio Virus Vaccine Live Oral Trivalent, Subpart B, Title 21 Code of Federal Regulations, April 1, 1996, 630.10-630.19.

Dr. B. Brock, Wyeth-Lederle Vaccines and Pediatrics, 401 N. Middletown Road, Pearl River, NY 10965-1299, U.S.A.

inants were present in the environment, they could be sources of infectious SV40. A summary of my thoughts on the implications of these hybrids for the polyomavirus workshop are on the next slide, please, on the right.

SV40 has the capacity to combine with unrelated viruses to produce new viruses with different biologic properties. It's theoretically impossible that SV40 could recombine with other viruses and be carried in humans as a recombinant.

Due to defectiveness of most the adeno-SV40 hybrids however, that have been isolated from monkey kidney-adapted adenoviruses, they lack growth advantages in human cells and it's unlikely that they are environmental contaminants. The current adenovirus vaccines are methodically tested and shown to be free of SV40. Thank you.

CHAIRMAN SNIDER: Thank you very much, Dr. Lewis. Could I ask if Dr. Brock from Praxis-Lederle is here?

DR. BROCK: Good afternoon. I'm Bonnie Brock from Wyeth Lederle. I've been asked to provide a brief overview regarding the quality control testing of the oral polio vaccine. I'd like to start by providing you with some product background on OPV.

The oral polio vaccine is a trivalent preparation of attenuated Sabin strains of polio virus types 1, 2, and 3 in an oral dosage form. The vaccine induces an immune response comparable to the natural disease. The vaccine is credited with the eradication and control of wild type polio in the United States.

Lederle Laboratories has distributed over 650 million doses since the licensure of Orimune in 1963. The viral content of the vaccine is specified by FDA regulations. The individual three polio virus types are combined in specific ratios to assure that all three stains immunize effectively.

The manufacture and testing of Orimune is a multi-stage process that's closely monitored by the FDA following explicit protocols and requires extensive quality control testing.

I'd like to describe cell culture preparation. Preparation of the cell substrate is in primary monkey kidney cells obtained from Green Monkeys that do not harbor the SV40 virus. The monkeys used as a source of kidney tissue are purpose-bred in isolated breeding colonies. They're tested for tuberculosis and viral antibodies. They're held in isolation quarantine under strict veterinary supervision.

A kidney perfusion process is performed under aseptic conditions which liberates kidney cells in preparation for cell culturating. Perfused kidneys are then delivered to the cell culture laboratory.

The cells are disbursed into monocellular suspensions under aseptic conditions. The cells are diluted into a growth media containing the nutrients necessary for growth and replication. Cells are planted into roller bottles and incubated to form a cell monolayer.

Cells are grown and observed for at least 11 days in the cell culture laboratory. After cell growth is completed, 75 percent of the roller bottles are sent to the virus production laboratory for polio virus inoculation. The remaining 25 percent of the roller bottles are sent to quality control for testing.

Fluids from all the roller bottles are tested to detect the presence of any transmissible, microbial agent by inoculation into four cells lines -- Cercopithecus monkey kidney cells, CMK cells -- for an initial 14 days, followed by a 14-day subculture, again in CMK; Rhesus monkey kidney cells for at least 14 days; rabbit kidney cells for at least 14 days; and BSC-1 cells for at least 14 days.

The 25 percent of all the cell culture bottles that are sent to quality control are then observed in their original control bottles for at least 14 more days, followed by a test to detect hemabsorptive viruses.

At day-4 of the quality control observation period, fluids are removed from the original bottles and again tested in the same cell systems I previously described. Again, to detect the presence of any transmissible microbial agent. We always include that additional 14-day subculture on CMK.

Again, at day-14 of the quality control observation period, fluids are again removed from the original bottles and again tested in those same cell systems, including a 14-day subculture in CMK. Therefore, every individual cell batch is observed for a total of more than 50 days in culture. The appearance of any sign of contamination at any stage of testing results in rejection of the cell batch.

I'd like to move on to virus production. One of the Sabin attenuated strains is prepared to inoculate production bottles. Master polio virus seed stocks are maintained in a viable state in liquid nitrogen storage.

Master viral strains have been prepared in the presence of SV40 virus neutralizing antiserum. All subsequent working seed strains have been prepared in CMK tissue and screened to assure they're free of SV40 virus.

The same level of virus is used for each group of bottles inoculated. Production bottles are examined and records checked. Only one polio virus type is processed at a time and incubated. At the appropriate time, post-polio virus infection, fluids from infected tissues which contain polio virus are harvested.

I'd like to describe viral harvest testing now. Viral harvest samples are sent to the quality control laboratory for evaluation and the rest of the harvested fluids are stored frozen until testing is completed. Fluids from these bottles are again tested to detect the presence of any transmissible microbial agent in CMK for 14 days, followed by a subculture in CMK for another 14 days.

Viral harvest fluids are also tested again in Rhesus monkey kidney cells, rabbit kidney cells, and BSC-1 cells, all for 14 days. Samples are also tested to demonstrate the absence of microplasma.

Quality assurance releases a virus harvest for further processing when all testing has been completed with satisfactory results -- for the original cell culture, the cell culture fluid testing and subcultures, and the viral harvest samples.

In summary, over 4,000 individual cell culture observations are made during the quality control testing of a single trivalent bulk lot. Any product contamination observed at any point, results in rejection.

When the appropriate number of harvests for a single polio virus type are released by quality assurance, they are thawed and combined to form a monopool. Samples from an unfiltered, prorata monopool are tested to ensure freedom from adventitious agents in rabbits, guinea pigs, adult mice, and newborn mice.

The production monopool is then passed through a .22 micron filter. Samples are taken for monopool testing by quality control to include testing for potency, testing for polio neurovirulence, testing for markers of attenuation. The appearance of any adventitious agent at any stage of testing results in rejection of the monopool. This process is repeated for each monopool virus type.

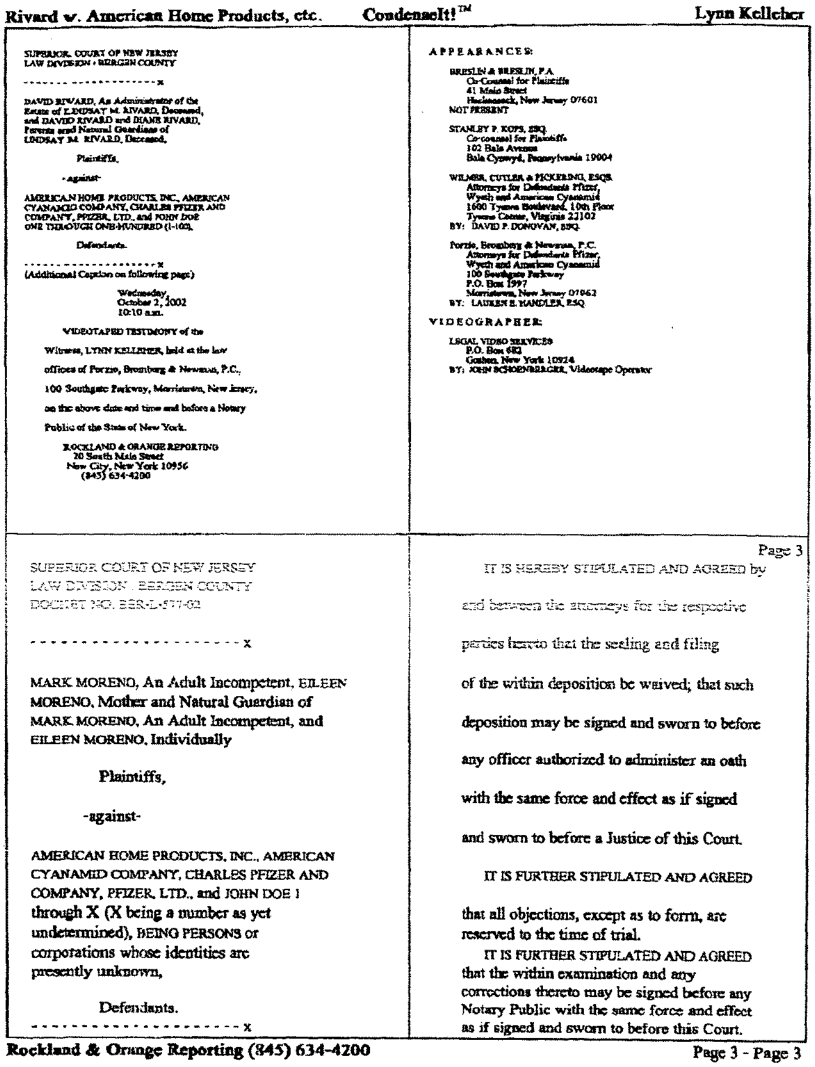
A document is then prepared containing the production history and test results on the monopool by quality assurance. This document is submitted to FDA Center for Biologics, Evaluation, and Research, along with monopool samples for testing. The FDA reviews the manufacture's test results, performs tests as appropriate, and provides notification of the release of the monopool for further manufacture.

Released monopools, one for each type, are combined with diluent to make a trivalent vaccine bulk preparation. Samples are tested by quality control for potency and sterility. The vaccine is aseptically filled into a single dose final containers. Samples are tested for quality control, for potency, identity, and safety. Final container samples are also sent to the FDA with a final protocol for the release of the final filled container vaccine for distribution.

And that completes my talk. Thank you for your attention.

CHAIRMAN SNIDER: Thank you, Dr. Brock, for that information. And now, Dr. Jim Williams from Pasteur-Merieux Connaught will talk about testing for SV40 and their viral vaccines. I believe we're going to use the overhead?

DR. WILLIAMS: Right. Thank you, Dr. Snider. We've heard a very detailed description from the previous speaker and since this is a presentation that we're concerned with SV40 infection, that's all we're going to talk about. We go through the similiar



Rivard v. American Home Products, etc. **CondenseIt!™** **Lynn Kelleher**

SUPERIOR COURT OF NEW JERSEY
LAW DIVISION - BERGEN COUNTY

- x

DAVID RIVARD, As Administrator of the
Estate of LINDSAY M. RIVARD, Deceased,
and DAVID RIVARD and DIANE RIVARD,
Parents and Natural Guardians of
LINDSAY M. RIVARD, Deceased,

Plaintiffs,

-against-

AMERICAN HOME PRODUCTS, INC., AMERICAN
CYANAMID COMPANY, CHARLES PFIZER AND
COMPANY, PFIZER, LTD., and JOHN DOE
ONE THROUGH ONE-HUNDRED (1-100),

Defendants.

- x
(Additional Caption on following page)

Wednesday,
October 2, 2002
10:10 a.m.

VIDEOTAPED TESTIMONY of the Witness, LYNN KELLEHER, held at the law offices of Porzio, Bromberg & Newman, P.C., 100 Southgate Parkway, Morristown, New Jersey, on the above date and time and before a Notary Public of the State of New York.

ROCKLAND & ORANGE REPORTING
20 South Main Street
New City, New York 10956
(845) 634-4200

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SUPERIOR COURT OF NEW JERSEY
LAW DIVISION - BERGEN COUNTY
DOCKET NO. BER-L-577-02

- x

MARK MORENO, An Adult Incompetent, EILEEN
MORENO, Mother and Natural Guardian of
MARK MORENO, An Adult Incompetent, and
EILEEN MORENO, Individually

Plaintiffs,

-against-

AMERICAN HOME PRODUCTS, INC., AMERICAN
CYANAMID COMPANY, CHARLES PFIZER AND
COMPANY, PFIZER, LTD., and JOHN DOE 1
through X (X being a number as yet
undetermined), BEING PERSONS or
corporations whose identities are
presently unknown,

Defendants.

- x

Page 3

IT IS HEREBY STIPULATED AND AGREED by and between the attorneys for the respective parties hereto that the sealing and filing of the within deposition be waived; that such deposition may be signed and sworn to before any officer authorized to administer an oath with the same force and effect as if signed and sworn to before a Justice of this Court.

IT IS FURTHER STIPULATED AND AGREED that all objections, except as to form, are reserved to the time of trial.

IT IS FURTHER STIPULATED AND AGREED that the within examination and any corrections thereto may be signed before any Notary Public with the same force and effect as if signed and sworn to before this Court.

Rockland & Orange Reporting (845) 634-4200 **Page 3 - Page 3**

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Lynn Kelleher

investigations as we went through the testing process if we believed that there was outside contamination of a sample of somehow.

Q. You mean -- but that would be a bacterial contamination. That wouldn't be a viral contamination.

A. It depends. It depends what it was.

Q. Between 1980 and 1987, when you came to the company until 1987, were there investigations when a harvest was rejected?

A. When I was down in that department?

Q. Yes.

A. No.

Q. Was that until 1990? Because you left that department you told me in 1990.

A. I left the department in 1990.

Q. Is that true that there were no investigations between 1980 and 1990?

A. Well, we may have started doing some preliminary investigations. Let's say if we had to reject material for non viral contamination, we may have done some investigations to see where the contamination was coming from.

Q. Bacterial, fungi, or mold?

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A. Yeah, right.

Q. But if it was viral, there would be no investigation; is that correct?

A. In the production bottles, that's correct.

Q. And who made that determination not to make an investigation?

MR. DONOVAN: Object to the form.

A. There was no need to do an investigation. If the viral -- if there was a viral agent in the roller bottles?

Q. Um-hum.

A. We decided -- we didn't do it because we didn't -- first of all, you don't want to open up that material in the laboratory and potentially infect everything else. And there was safety issues handling that material. So as soon as you saw the observation in the roller bottle that there was some sort of viral contamination, we immediately decontaminated the bottles and got rid of them.

Q. What happens if there was less than eighty percent survival --

A. Unless the --

Q. -- and -- and no cytopathology?

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A. Well, depending on the total number of bottles that came down with degeneration because I told you it could be caused by other things other than contaminants and whatever, we would go ahead and test that material and in some instance we may have sent samples out for microbial contamination identification.

Q. You had a serology department at American Cyanamid, correct?

A. Yes.

Q. Is that part of department -- of the in vivo department?

A. No, that was part of the in vitro department, also.

Q. So that was part of your department?

A. Um-hum.

Q. Is that yes?

A. Yes.

Q. And you were the supervisor of that department?

A. Yes.

Q. From 1980 to the year --

A. '81, late '81.

Q. Late '81 to the year 1990; is that

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correct?

A. To the beginning of 1990.

Q. Did you ever see a serological test conducted to determine whether or not a African Green monkey was infected with SV-40?

A. I may have done -- it may have been done for investigative purposes only.

Q. Once or twice?

A. Yeah, a couple times.

Q. But it was never done as a matter of routine; is that correct?

A. Correct.

Q. Between 1990 and the year 2000, am I correct that it was also not performed as a matter of routine?

A. On the monkey material?

Q. Yes.

A. On the actual monkey serum?

Q. Correct.

A. Correct.

Q. Now, you did, however, test on the monkey serum for foamy virus; is that correct?

A. Yup.

Q. Yes.

GOOD MANUFACTURING PRACTICE GOOD LABORATORY PRACTICE STANDARD OPERATING PROCEDURE	NUMBER 122-616	PAGE 1 OF 11
	SUPERSEDES 4.2.79	DATE ISSUED Revised 11/6/83
SUBJECT STANDARD OPERATING INSTRUCTIONS	WRITTEN BY S. Dubpernell	
REFERENCE GOOD LABORATORY PRACTICE AND GOOD MANUFACTURING PRACTICE REGULATIONS–FEDERAL REGISTER VOL 43, NO 247 AND NO 19[illegible] RESPECTIVELY	DEPARTMENT NAME Virological Testing (Dept. 814)	
PURPOSE Poliovirus Tissue Culture Safety Tests		
RESPONSIBLE Ms. S. Dubpernell	APPROVAL Dr. T. Ubertini	

Scope and Procedure - See attached sheets.

601017

S.O.I. POLIOVIRUS VACCINE TISSUE CULTURE SAFETY TESTS

b0J0J8

SCOPE: Inoculation of spent growth fluids, production control bottle fluids and viral harvests into various tissue cultures selected to detect the presence of any adventitious viral agents attributable to the vaccine substrate.

A. Test Samples

1. Spent Growth Fluids (SgF) constitute the growth medium removed from the poliovirus production bottles immediately prior to the seeding of those vessels with virus. The fluids are collected and dispensed into testing aliquots in Dept. 472 and delivered to Dept. 814 where they are stored below -20°C until tested.
2. Production Control Bottle fluids (PCB-1) are those fluids collected in Dept. 814 from the 25% Production Control Bottles on the day of viral harvest. They are dispensed into testing aliquots (10 ml) and stored below -20° until tested. See S.O.I. 122-591, "Handling of Poliovirus Production Control Bottles."
3. Production Control Bottle Fluids (PCB-2) are those fluids collected in Dept. 814 from the 25% Production Control Bottles no less than 14 days after seeding of the production vessels with poliovirus. The fluids are dispensed into testing aliquots (10 ml) and stored below -20°C until tested. See S.O.I. "Handling of Poliovirus Production Control Bottles". #122-591.
4. Vessels representing 75% of the cell suspension from the kidneys from each monkey are seeded with poliovirus and the viral fluids pooled there from are considered to be a single harvest. Harvest test samples are submitted from the Production Department to the testing department where they are stored below -20°C until tested.

B. Tissue Cultures for Safety Tests

1. Primary rhesus monkey kidney cell monolayers (RMK). RMK cultures are used to screen for the presence of simian agents such as foamy viruses, adenovirus, SV5 and other viruses causing a cytopathic effect (CPE).

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2. Primary cercopithecus monkey kidney cell monolayers (CMK). CMK cultures are used to screen for the presence of vacuolating agent (SV40), cytomegalovirus and other herpesviruses, foamy viruses and other viral agents capable of producing a CPE.
3. Primary rabbit kidney cell monolayers (RbK). RbK cultures are used to screen primarily for the presence of Simian B virus.
4. BSC-1 (Bureau of Standards cercopithecus -1) cell strain is used to screen for the presence of measles virus. In addition, a 10 ml aliquot of the PCB-2 fluids from each harvest is tested for the presence of rubella virus by the interference procedure using Echovirus 11 challenge.
5. Lederle 130, human diploid fibroblast cell strain. Lederle 130 is used to screen for the presence of cytomegalovirus in particular, but is susceptible to other simian viruses as well.

   All cell cultures are grown in 75 cm^2 plastic culture flasks (PCF) in Dept. 472. RbK cultures are grown in 150 cm^2 plastic flasks (T-150 or CPF 150) or in Blake bottles as well. All cultures are grown to confluency of monolayer and are renewed with the appropriate maintenance medium, 15 ml/PCF, 50 ml/larger vessel, before shipment to the testing area.

C. Maintenance Medium

Refer to S.O.I. 122-574 for Preparation of Maintenance Media.

1. RMK/CMK Earle's lactalbumin hydrolysate maintenance medium, 0.16% sodium bicarbonate. (Eala, 0.16% bicarb)
2. RbK Eala, 0.22% bicarb plus 3% fetal calf serum (FCS)
3. BSC-1/Lederle 130 Minimal Essential Medium (Eagle), 0.16% sodium bicarbonate, 2% fetal calf serum (MEM-E, 2% FCS), or Earle's lactalbumin maintenance medium, 0.16% sodium bicarbonate, plus Earle's balanced salt solution and 2% FCS.

   An additional low percentage (1-2%) of fetal calf serum may be added to the maintenance medium at the time of fluid renewal if the condition of the cultures warrant it.

D. Materials/Equipment

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Flasks and bottles with bells for dispensing maintenance media into workable volumes.
pipettes: 10 ml and 25 ml serological
pipetting aids
36° (± .5°C) incubator
inverted microscope
storage freezer, mechanical or liquid nitrogen chests
assorted size flasks or bottles for diluting antisera
sterile capped vials in trays obtained from Sterile Filling, assorted sizes for test sample storage
test tissue cultures
37°C (± .5°C) waterbath
10 ml evacuating pipettes and vacuum source

E. Procedure for Safety Test - Non-Harvest Material

1. Ten ml of SgF, PC5-1 and PCB-2 fluids are tested in each of the four tissues, RMK, CMK, RbK and BSC-1. The samples are thawed immediately prior to test.
2. Ten ml of sample is inoculated into the corresponding PCF properly labelled with sample identification and test date.
3. Tissue culture controls, inoculated with 10 ml of the appropriate maintenance medium are included for each test.
4. All cultures are incubated at 36° ± .5°C.
5. All test cultures and controls are examined microscopically before fluid renewal at approximately day 7. The fluids are aspirated aseptically and replaced with the appropriate fresh maintenance medium, 25 ml/PCF.
6. The cultures are returned to the incubator for a total of 14 days incubation. A final microscopic evaluation is made at that time.
7. All CMK tests are subcultured for an additional 2 weeks by regulation. See Subculture Procedure following. Freeze CMK test cultures and controls below -20°C until subculture is performed.

8. Two additional tests are performed on the PCB-2 fluids:

   a. Following final microscopic evaluation of the PCB-2 fluids tested in BSC-1, both sample and control cultures are tested for the presence of rubella virus by the interference method. The test and control cultures are evacuated and overlaid with a 20 ml aliquot of ECHO-11 virus diluted in maintenance medium calculated to contain 1000 to 10,000 TCID50 challenge. Cultures are incubated at 36°C (±.5°C) for 3 additional days before microscopic examination for Echovirus cytopathology. If Echovirus CPE is observed, rubella virus is considered to be absent.

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   b. Ten ml PCB-2 fluids are inoculated into a PCF of Lederle 130 tissue. Test samples and tissue culture controls are incubated for a total of 28 days at 36°C (±.5°C). Microscopic examination and fluid renewals are made at weekly intervals. Final microscopic evaluation is made on day 28.

F. Procedure for Harvest Safety Test - RMK, CMK, and HuA

1. According to the Code of Federal Regulations, Part 21, §630.16(a) 5, 6, 7..." at least 500 doses or 50 ml whichever represents a greater volume of virus, of each undiluted monovalent virus pool, or in equal proportions from individual harvests or subpools, shall be tested"...in the required cultures except for rabbit cultures in which at least 500 ml must be tested.
2. Examples: At the present time, harvests are tested on the basis of a planned 2-harvest monopool.

   $\frac{50 \text{ ml}}{2}$ = 25 ml to test in each RMK, CMK and BSC-1

   In rabbit tissue: $\frac{500 \text{ ml}}{2}$ = 250 ml test volume.

3. The minimum test volume is 10 ml in RMK, CMK and BSC-1, 100 ml in RbK, independent of how many harvests are pooled together to make a monopool. b07022
4. RMK, CMK and BSC-1 safety tests: the poliovirus harvest sample must be neutralized prior to test with its homotypic antiserum diluted to use - dilution.
5. The antisera presently in use are:

Anti-Type I	Horse	6083	several bleeding dates
		6084	
		6085	
		6086	
Anti-Type II	Horse	6087	several bleeding dates
		6088	
Anti-Type III	Horse	6089	several bleeding dates
		6090	
		6091	
		6092	

6. The anti-sera were prepared in 1978 by hyperimmunization of horses. The antigens used were prepared in Hep-2 cell cultures. All horses were bled several times before final bleed out to produce stock antiserum. The antiserum is stored in Dept. 814 below -20°C.

   All antisera are inactivated for 30 minutes in a 56°C waterbath. Inactivation is done once before initial use of the working stock supply and does not have to be repeated before each test.
7. The level of poliovirus antibodies in the particular "bleeding" used for test must be determined prior to use against an undiluted harvest challenge. (See Procedure for Determination of Poliovirus Antibodies in Immune Serum Globulin #122-598.)

   The antiserum is used for test purposes at a dilution that is 4 times the concentration of its antibody titer. For example, if the antibody titer is 1:300, the antiserum would be diluted 1:75 for test use.

8. Equal volumes of harvest and type specific antiserum at use dilution are mixed and incubated for 2 hours in a 37°C (±.5°C) waterbath.
9. A serum control is prepared by combining 10 ml diluted antiserum plus 10 ml maintenance medium and neutralized along with the samples.
10. After neutralization, the 50 ml neutralized test sample is inoculated into 3 PCF's of test cultures, 20 ml, 20 ml, 10 ml. The 20 ml of serum control is inoculated into a single PCF.
11. The test is incubated for 14 days at 36°C (±.5°C). Microscopic examination and fluid renewals are carried out as for PCB fluids above.

    A small percentage of type-specific antiserum is included in the renewal medium, 0.5% to 2%, depending on the antibody titer.
12. Final microscopic evaluation is made on day 14. All CMK test cultures, harvest, serum control and tissue controls, are frozen for subculture.

G. Harvest Test in Rabbit Cell Cultures

1. Poliovirus harvests do not require neutralization before test in RbK cultures because the virus does not produce a cytopathic effect in that system.
2. The 250 ml test sample is divided between 5 Blake bottles or plastic 150 flasks, 50 ml per container.
3. Tissue culture controls, 50 ml maintenance medium/vessel are included in the test.
4. All cultures are incubated for 2 hours at 36° (± .5°C) after which the fluids are aspirated and the cultures renewed with fresh maintenance medium, 100 ml per container. (Raw harvest is toxic to the RbK cultures and should be removed after a 2 hour adsorption period.)

5. The test is incubated for 14 days at 36°C (± .5°C) with fluid renewal again about the 7th day.

6. Final microscopic evaluation is made on the 14th day.

H. Criteria for Evaluation

1. The purpose of the tissue culture safety test is to detect any adventitious agents that may be present in the vaccine substrate. When final test evaluations are made, it is important to determine the actual source of the contaminant from the test sample or present in the test tissue which may itself harbor an agent.
2. Retests and subcultures are scheduled by the evaluator on the following basis:

Basis #1

Sample		TC Control	
7	14	7	14
0	+	0	0

Requires: subculture and retest.
If subculture is +, a second retest is required.

NOTE: + may refer to any type of cytopathology, such as FV, RC CPE, or to non-specific degeneration (D) or to sheet sloughing (SS).

Basis #2

Sample		TC Control	
7	14	7	14
0	+	0	+

Same appearance as control.
Invalid test. The agent is in the test tissue.
Requires: retest only.

Basis #3

Sample		TC Control	
7	14	7	14
0	0	0	0

Subculture :

Subc. Sample		Subc. TC Control		TC Control	
7	14	7	14	7	14
0	+	0	0	0	0

Requires: 2 retests with subculture

Basis #4 b07025

Sample		TC Control	
$\frac{7}{0}$	$\frac{14}{+}$	$\frac{7}{0}$	$\frac{14}{0}$

Subculture:

	Subc. Sample		Subc. TC Control		TC Control	
	$\frac{7}{0}$	$\frac{14}{+}$	$\frac{7}{0}$	$\frac{14}{0}$	$\frac{7}{0}$	$\frac{14}{+}$
or	$\frac{7}{0}$	$\frac{14}{+}$	$\frac{7}{0}$	$\frac{14}{+}$	$\frac{7}{0}$	$\frac{14}{0}$

Same appearance as control.

Invalid test because agent is in test tissue.

Requires: resubculture

NOTE: Basis #3 and Basis #4 refer to CPK safety tests which are routinely subcultured by regulation.

Basis #5

Sample		TC Control	
$\frac{7}{0}$	$\frac{14}{+}$	$\frac{7}{0}$	$\frac{14}{0}$

Subculture:

Subc. Sample		Subc. TC Control		Subc. TC Control	
$\frac{7}{0}$	$\frac{14}{0}$	$\frac{7}{0}$	$\frac{14}{0}$	$\frac{7}{0}$	$\frac{14}{0}$

Agent not transmissible.

Requires: one retest. (For CPK the retest will be subcultured as well).

3. Any number of other situations may arise. These are discussed with the supervisor or other responsible personnel and a value judgement made at that time.
4. Tests contaminated with non-viral agents, such as yeast, mold or bacteria, are retested. In certain cases, where no cell damage is observed, antibiotics may be added to the contaminated culture in an attempt to save the test. (Refer to S.O.I. 122-566. Use of Antibiotics to Control Non-Viral Contamination in Tissue Culture Safety Tests.)

I. Subculture Procedure

1. All CMK safety tests and appropriate controls are routinely subcultured by regulation, with the exception of those deemed invalid by Basis #2 above.
2. Other tissue culture safety tests are subcultured as determined by the final evaluation.
3. Cultures for subculture are held frozen until tested. The cultures are thawed and 10 ml of the fluids inoculated into a fresh PCF of the appropriate tissue. Tissue culture controls are subcultured as well.
4. Harvest subcultures require reneutralization of the samples and serum controls before inoculation as described in Section F 5-12 above.
5. Subcultures are incubated for 14 days 36°C (±.5°C). Microscopic evaluation and fluid renewals are made as for the primary safety tests.
6. Final microscopic evaluation is made on day 14. Criteria for evaluation are the same as in Section H above.
7. Any adventitious agent attributable to a harvest sample, spent growth or control bottle fluid is cause for rejection of the lot from vaccine use.
8. The thawed test material remaining in the PCF is transferred to a sterile vial and returned to freezer storage for any necessary resubculture.

J. Record Keeping

1. All record of all tests, dates performed and test results are kept in a loose leaf notebook entitled "Polio Testing" in the test laboratory - in summary form only.

2. Every tissue culture safety test for every sample on every cell culture has an individual work sheet on which is detailed all pertinent data:
    - sample number
    - tissue identification number
    - S.O.P. number
    - date tissue made
    - type test container
    - date sample received
    - storage temperature of sample
    - test date
    - test performed by (initials)
    - incubator number
    - incubation temperature
    - neutralization procedure, if applicable
    - date renewed
    - medium used, lot number
    - antiserum used
    - renewed by (initials)
    - volume sample tested per container
    - microscopic evaluations, date made and reading
    - evaluator - initials
3. The final evaluation and disposition of the test (pass, reject, subculture, etc.) is stated.
4. Two signatures are present - that of the final evaluator and that of a responsible appraiser.
5. These work sheets are bound into workbooks and are filed in the office where they become a permanent record.

S. Dutremell

Approved By:

RESULTS OF TESTING PRODUCTION LOTS OF ORAL POLIOVIRUS VACCINE

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During the past 4 years these laboratories have produced and tested Sabin strains of oral poliovirus vaccine in accordance with the requirements of the Public Health Service (1961). The tests conducted on the vaccine are described, and the results of the tests are reported in this paper.

METHODS

More than 1200 monkeys, 349 *Macaca mulatta* (rhesus) and 865 *Cercopithecus ethiops*, were used for poliovirus pro-luction in the period 1960–1964. These nonkeys were isolated soon after trap-ing by a method described by Vickers 1 and shipped to Pearl River, New ork. An intrapalpebral tuberculin test d a serological test for simian foamy us antibodies were performed on each onkey at the beginning of the isolation eriod. Groups of approximately 50 nimals each were isolated in individual ges. After a 6-week period they were crificed and their kidneys removed for sue culture preparation.

Tissue cultures derived from the dneys of 1 monkey were inoculated th 1 type of poliovirus, and the vac-e produced was called a *monovalent cine harvest*. This was tested accord- to Public Health Service require nts, as shown in figure 1. Monovalent cine harvests that were found satis-tory were pooled, called *monovalent cine pools*, and tested according to Public Health Service requirements (figure 2).

Monovalent vaccine pools that passed all required tests were diluted to the appropriate dose form and filled into vaccine containers. Further tests were conducted at this stage, as shown in figure 2. All tests are outlined in the Public Health Service requirements except the 2 which follow.

Virus potency test.—Poliovirus concentration was measured by serially diluting a sample to extinction in half-log steps. Two-tenths ml of each dilution were inoculated into 10 tubes containing rhesus monkey kidney (RMK) tissue culture and 1.8 ml of maintenance medium per tube. A reference virus was titrated in the same manner. After inoculation the tubes were incubated at 36 C and examined for cytopathology at the end of a 7-day period. The 50% end point of infectivity ($TCID_{50}$) was determined by the method of Reed and Muench (1938).

Virus identity test.—Equal volumes of type-specific antisera and virus test sample were mixed. After 1-hour incubation at 37 C the mixture was inoculated into tubes of RMK tissue. After 7-day incubation at 36 C the test cultures and appropriate controls were examined microscopically for poliovirus cytopathology.

RESULTS

Monkey group mortality and morbidity.—Table 1 indicates the number of monkeys isolated and used for vaccine production. Monkeys isolated but not

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EXHIBIT
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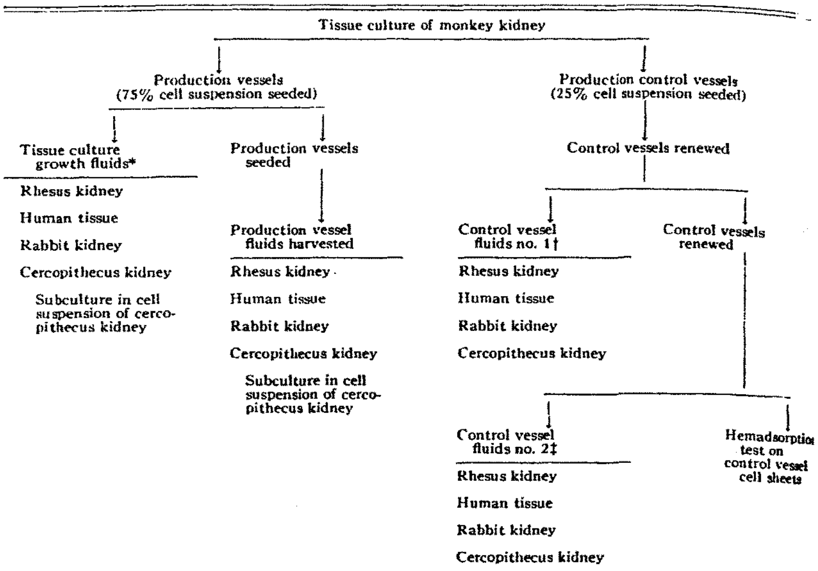
FIGURE 1.—Test scheme for monovalent vaccine harvest


Tissue culture of monkey kidney
Production vessels (75% cell suspension seeded)
Production control vessels (25% cell suspension seeded)
Tissue culture growth fluids*
Rhesus kidney
Human tissue
Rabbit kidney
Cercopithecus kidney
Subculture in cell suspension of cercopithecus kidney
Production vessels seeded
Production vessel fluids harvested
Rhesus kidney
Human tissue
Rabbit kidney
Cercopithecus kidney
Subculture in cell suspension of cercopithecus kidney
Control vessels renewed
Control vessel fluids no. 1†
Rhesus kidney
Human tissue
Rabbit kidney
Cercopithecus kidney
Control vessels renewed
Control vessel fluids no. 2‡
Rhesus kidney
Human tissue
Rabbit kidney
Cercopithecus kidney
Hemadsorption test on control vessel cell sheets

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* Pooled fluids taken from production vessels immediately prior to virus inoculation.
† Pooled fluids taken from control vessels at time of virus harvest.
‡ Pooled fluids taken from control vessels 14 days after viral inoculation of production vessels.

employed for vaccine production were used for the preparation of tissue cultures for testing purposes.

Mortality in terms of monkeys isolated was approximately the same (3%) for both groups of monkeys, with enteritis the primary finding on necrop for rhesus monkeys and pneumonia cercopithecus monkeys (table 2). E dence of tuberculosis was rare in bo groups of monkeys. The incidence tuberculin-positive skin reactors pr

FIGURE 2.—Test scheme for monovalent vaccine pools

Vaccine stage	Process step	Test	Animal
Undiluted vaccine pool	Nonfiltered pool	Tests for safety: B virus Lymphocytic choriomeningitis virus Coxsackie virus *Mycobacterium tuberculosis*	 10 rabbits 20 adult mice 20 suckling mi 5 guinea pigs
	Filtered pool	Neurovirulence tests in monkeys: Intracerebral Intraspinal In vitro marker tests: ~~*t* Marker~~ *d* Marker Identity Virus assay	 30 monkeys 15 monkeys
Diluted vaccine pool	Diluted bulk vaccine pool	Virus assay Identity Sterility	
Final vaccine container	Filled	Virus assay Identity Sterility Safety tests	 2 mice 2 guinea pig

TABLE 1.—Monkey mortality in isolation groups

Type of monkeys	Number of monkeys isolated	Number of monkey groups	Number of monkeys used for vaccine	Number of deaths
Rhesus	830	32	349	28
Cercopithecus	2184	88	865	70

TABLE 2.—Primary monkey necropsy findings

	Rhesus	Cercopithecus
Pneumonia	3	43
Pneumonia and enteritis	3	6
Enteritis	15	11
Acute gastritis	1	1
Multiple organ abscesses	1	1
Granulomatous disease	1	
Encephalitis*	1	
Tuberculosis	1	1
Peritonitis	1	
Accidental death	1	5
Intussusception		1
Focal hepatitis		1
Totals	28	70

* Microscopic findings.

o isolation was extremely low, and ubercular lesions were found on ne- ropsy in only 2 monkeys.

onovalent vaccine harvest.—The re- s of the tissue culture safety tests nducted on the vaccine derived from h individual animal are summarized able 3. Fifty-six per cent of the rhe- monkey vaccine harvests were re- ted because of the occurrence of ventitious viruses. Viruses producing cytia, including simian foamy and ian measles, were responsible for proximately half the rejections, while uolation suggestive of SV_{40} virus was nd in the other half of the rejected cine harvests. Only 1 vaccine harvest tained an agent that caused a cyto- type of cytopathology (CPE), and agent was not identified. On 2 occa- s a hemadsorption agent was found in vaccine harvests produced from rhesus monkeys.

Thirty-six per cent of the cercopithecus monkey vaccine harvests were rejected because of the presence of adventitious viruses. Viruses producing a syncytial type of cytopathology accounted for 303 of the 309, or 98% of those rejected. Seventy-five of the 303 were serologically identified as follows: 50 simian foamy virus, 25 simian measles virus. No evidence of SV_{40} virus was found in the 865 monkeys examined. An agent causing a cytolytic effect was demonstrated on 6 occasions; 1 of the 6 isolates was serologically identified as an adenovirus, and no attempt was made to identify the other 5. Later studies indicated that adenoviruses accounted for the majority of viruses causing cytolytic CPE in cercopithecus monkey kidney (CMK) tissue cultures handled in this laboratory. On 1 occasion a hemadsorption virus serologically identified as SV_5 was isolated from a monovalent vaccine harvest from a cercopithecus monkey.

As to sensitivity of the tissue culture systems in detecting viral cytopathology, tables 4 and 5 demonstrate that the primary CMK tissue culture test system appears to be more sensitive in detecting adventitious agents in both RMK and CMK tissue cultures. However, on 2 occasions during the testing of RMK tissue cultures agents were isolated that were not detectable in the CMK tissue culture test system. One was a hemadsorption virus which was detectable only in a RMK tissue culture system,

TABLE 3.—The occurrence of viral cytopathology (CPE) in monkey kidney tissue cultures

onkey type	Number of vaccine harvests		Type of CPE		
	Tested	With CPE	Syncytial	Vacuolating	Cytolytic
	349	196 (56%)	91 (46%)	104 (53%)	1 (<1%)
thecus	865	309 (36%)	303 (98%)	0	6 (2%)

TABLE 4.—Detection of viral cytopathology (CPE) in kidney tissue cultures from 196 rhesus monkeys

Vaccine level tested	Number of tests with viral CPE			
	CMK*	RMK	RbK	Hu
Tissue culture growth fluids	80 (41%)	18 (9%)	4 (2%)	3 (2%)
Vaccine harvests	80 (41%)	21 (11%)	6 (3%)	2 (1%)
Control fluids 1	105 (53%)	42 (21%)	28 (14%)	14 (7%)
Control fluids 2	147 (75%)	68 (35%)	67 (34%)	48 (24%)

* Tissues culture test system: CMK, cercopithecus monkey kidney; RMK, rhesus monkey kidney; RbK, rabbit kidney; Hu, human tissue culture.

TABLE 5.—Detection of viral cytopathology (CPE) in kidney tissue cultures from 309 cercopithecus monkeys

Vaccine level tested	Number of tests with viral CPE			
	CMK*	RMK	RbK	Hu
Tissue culture growth fluids	61 (18%)	5 (<2%)	4 (1%)	14 (4%)
Vaccine harvests	15 (5%)	13 (4%)	2 (<1%)	15 (5%)
Control fluids 1	135 (44%)	29 (9%)	44 (14%)	30 (10%)
Control fluids 2	222 (72%)	88 (28%)	105 (34%)	443 (14%)

* Tissue culture test systems: CMK, cercopithecus monkey kidney; RMK, rhesus monkey kidney; RbK, rabbit kidney; Hu, human tissue culture.

and the other was a syncytial agent detectable in rhesus, rabbit, and human kidney tissue cultures but not in the CMK tissue culture systems.

In testing CMK tissue cultures agents isolated on 5 occasions were not detectable in the CMK tissue culture test system. Two of these agents were serologically identified as simian foamy viruses, while 3 were unidentified syncytial agents. Three of the 5 detections were made in RMK tissue culture test systems and the other 2 were in human kidney tissue culture test systems.

As would be expected, the second sample (figure 1, footnote ‡) of the control bottles was the most sensitive te level. In 1963 the CMK tissue cultu safety test system was expanded include a subculture at the tissue cultu growth fluid and vaccine harvest level Over 450 CMK-produced vaccine ha vests were tested in this manner, and was found that the subcultures increased the percentage of detectio approximately 50% in both the tissu culture growth fluids and in the vaccin harvest.

Monovalent vaccine pools.—Laboratory animals.—Nonfiltered vaccine samples of pooled harvests were inoculate into small laboratory animals for th detection of simian B virus, lymphocytic choriomeningitis virus, Coxsacki virus, and *Mycobacterium tuberculosis* None of these agents were demonstrate in any vaccine pools. A summary of th results of the small animal tests is sho in table 6. The animal mortality was no instance due to the vaccine.

Genetic markers.—The following test for genetic stability of the vaccine wer conducted on filtered samples: monke neurovirulence, *t* marker, and *d* marke The results of the neurovirulence test will be published in a separate pape

t Markers (Lwoff and Lwoff, 1959 Public Health Service, 1961): Attenuated poliovirus strains which gro readily at 40 C are classified as *t*-positiv in contrast to *t*-negative strains whic show an increased growth at 36 C of a least 100,000-fold over that obtained a 40 C. The *t* marker was consistent in al 3 types, with each vaccine pool havin a negative *t* marker. The differen between the viral growth at 36 C an

TABLE 6.—Results of tests in laboratory animals

Animal	Test	Number of animals used	Mortality number	Primary cause of mortality
Rabbit	B virus	1490	8 (0.5%)	Enteritis
Guinea pig	Lymphocytic choriomeningitis virus and *Mycobacterium tuberculosis*	745	23 (3%)	Pneumonia and enteritis
Adult mice	Lymphocytic choriomeningitis virus	2980	27 (0.9%)	Cannibalism and enteritis
Suckling mice	Coxsackie virus	2980	144 (4.8%)	Cannibalism and enteriti

0 C was at least 5 logs for each vaccine ot.

d Markers (Dulbecco and Vogt, 1958; Public Health Service, 1961): Attenu-ted poliovirus strains which grow eadily at low concentrations of bicarbo-ate are classified as *d*-positive in con-ast to *d*-negative strains which exhibit elayed growth under the same condi-ons. The *d* marker was also stable for ach vaccine type. All vaccine pools had negative *d* marker.

Virus assay.—Two vaccine dosage forms were produced: a concentrated vaccine containing 0.1 ml per dose, and diluted vaccine containing 2 ml per dose. Vaccine pools were diluted to contain 300,000 $TCID_{50}$ per dose. The assay method was designed to allow for the rapid testing of samples with good reproducibility. To determine the main source of assay error an experiment was designed to show the part contributed the technician, the method, and the individual animal tissue. The main variable was found to be the individual animal tissue. This could be reduced by using tissues from separate animals for individual titrations and by either aver-ging the results of several assays or adjusting the titer to a standard test reference.

The standard deviation for 1 potency test was 0.16 log with a 95% confidence interval of ±0.3 log. Duplicate virus titrations were performed during each of at least 3 stages of vaccine preparation.

Identity test.—This test was performed after each dilution or after each manipulation that required a change in the vaccine container. In approximately 2000 identity tests conducted, on only 1 occasion did a monovalent vaccine contain a poliovirus in addition to the prescribed type. In this instance the vaccine pool was discarded. It was not established whether the sample contamination occurred in the production laboratory or in the testing laboratory.

Sterility test.—Although tests for microbial sterility are conducted at several stages of vaccine production, the only required sterility test is on the monovalent bulk vaccine. A review of the records indicates that tests were conducted on more than 1200 vaccine harvests with fewer than 0.5% contaminated. Bacterial or mycotic contamination was observed in 3 vaccine pools and in 3 final vaccine lots which were subsequently rejected.

Safety test.—As a final safety test vaccine from each filling operation must be tested in guinea pigs and mice. Four hundred sixty-five individual safety tests conducted on final vaccine fillings have proved satisfactory. No vaccine filling has produced any untoward effect in the test animals.

DISCUSSION

Oral poliovirus vaccine is probably the most thoroughly tested biological product every produced. To prepare this vaccine successfully a rigid testing schedule begins with the procurement of the animals to be used for vaccine production and proceeds until all testing requirements are met. An interval of approximately 6 months is required to complete the testing program on each vaccine lot. Tissue culture safety test systems are used which offer optimum sensitivity in detecting adventitious agents of either human or monkey origin (Hull and Minner, 1958; Sweet and Hilleman, 1960; Cabasso et al, 1959). The high incidence of SV_{40} virus in the rhesus monkey made it undesirable as a source of tissue for vaccine production. Although monkeys that were not infected could be selected by serological screening, it seemed more practical to use a monkey not harboring this agent. The cercopithecus monkey is a readily available animal well suited for vaccine production. The major problem encountered with this monkey has been the occurrence of simian foamy virus which at present appears to be a monkey orphan virus. Although the overall rejection rate has been 36%, with foamy virus probably accounting for two-thirds, the rejection rate in the past year has been reduced to less than 10% by careful selection and isolation.

Since only monkeys negative for simian foamy virus antibodies were used for vaccine production, the occurrence of this virus in tissue cultures from such animals may be somewhat lower than in untested animals. The failure of the serological test to detect all animals with simian foamy virus may be due to the fact that recent infection had occurred and antibodies had not yet had time to develop, or possibly to the fact that the antigenic spectrum of the test was incomplete.

The 6-week isolation period reduced the incidence of some viruses and probably accounts for the fact that no simian B virus and very few hemadsorption viruses have been isolated from the large number of monkeys processed.

The variety of adventitious viruses found in cercopithecus monkeys handled by the method outlined in this paper was relatively small. The main isolates were simian foamy virus, simian measles virus, and occasionally an adenovirus and a hemadsorption virus.

The test systems used appeared to be adequate, since additional systems did not add significantly to the sensitivity of these tests. Longer test periods (21 days) and 1 additional subculture for a 21-day period were also tried with little change from results of the present tests. The efficacy of the tissue culture safety test was confirmed by the fact that no agents such as B virus were detected in later stages of testing in laboratory animals.

The results of the genetic ma tests indicate that monovalent vac pools prepared from all 3 poliovi types remained stable during the prod tion period. Tests on the vaccine in fi containers were designed to add furt proof of the vaccine's safety.

The basic requirements est by the Division of Biologics Stand of the Public Health Service ha proved both thorough and effective insuring vaccine safety, purity, an potency.

SUMMARY

The results of testing oral poliovir vaccine produced over a 4-year peri are reported. The occurrence of adve titious viruses in rhesus and cercopith cus monkey kidney tissue cultures an the comparison of various test syste are discussed. The requirements esta lished by the United States Publ Health Service have proved both tho ough and effective in determining t safety, purity, and potency of th vaccine.

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INTEROFFICE CORRESPONDENCE

E&D NOV 8 19[illegible]

Polio Vaccine Testing 11.8.61

TO:

ATTN. OF: Dr. I. S. Danielson

COPY TO: Dr. H. D. Plumme[illegible]
Mrs. M. Priestley
File

SUBJECT: Presence of SV40 in Vaccine Lots

REFERENCE:

In view of the recent emphasis that the NIH has placed on the detection of SV40 in vaccine lots, the following is a summary of the incidence of SV40 found at the PCB-2 level of the fifteen lots released for clinical trial.

Lot No. 114
Lot No. 216
Lot No. 317

As you know the PCB-2 level is a subculture of at least 25% of the kidney cultures used for polio virus production. The harvest samples were negative for SV40 in our laboratory, but no subculture was made. It is entirely possible that SV40 might be found in a subculture of the original harvest fluids. The decision by Dr. Murray to allow SV40 to be present at the PCB-2 level was the basis for our allowing these lots to pass. Would it be wise in asking the NIH to allow us to substitute three new lots in place of those outlined above? This is particularly important on Lot 317 which shows and increased neurovirulence.

I believe we should also consider a new emphasis on cercopithecus monkey for production purposes. According to Dr. Gerber, practically 100% of these monkeys are free of SV40. Our results indicate that SV40 is found in about 10% of the cercopithecus monkey kidneys harvested at Lederle.

James L. Bittle

JLB:amb

704374

PENGAD-Bayonne, N.J.
PLAINTIFF'S EXHIBIT NO. 31
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DATE 10-2-02 RPTR:

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ONCOGENIC VIRUSES

THIRD EDITION
COMPLETELY REVISED AND ENLARGED
IN TWO VOLUMES

by

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CHAPTER 23

Oncogenic Potency of Simian Virus 40 (SV 40)

Monkey kidney cell cultures have been used extensively in recent years for the propagation of certain viruses, such as poliomyelitis or adenoviruses, particularly for the preparation of vaccines. Cell cultures prepared from kidneys of thousands of monkeys have been employed by pharmaceutical companies engaged in the production of such vaccines.

Ideally, a cell culture employed for the propagation and subsequent harvesting of a virus should be free from other bacterial or viral agents; this is particularly imperative in case such cultures are to be used for the preparation of live vaccines, such as the Sabin oral poliomyelitis vaccine. This condition, however, is difficult to fulfill; no cell cultures are entirely free from indigenous viruses, particularly if these agents are latent and not detectable under the usual laboratory conditions. Certain viruses may multiply in a cell and yet may not cause any degenerative changes in cell morphology; such viral agents are obviously very difficult to detect. Other viruses are cytopathogenic and cause characteristic degenerative changes in the cells in which they are propagated.

Attempts to Detect Latent Viruses in Tissue Culture. Several methods are employed in order to detect latent viruses. To begin with, a virus which is initially latent may become cytopathogenic following serial passage in the same type of cells. In another attempt to detect a latent virus, the fluid collected from tissue culture in which a latent virus is presumably present can be transferred to a cell culture of another type. This is done in order to determine whether a virus latent in one type of cells may not induce degenerative changes in another cell variety, perhaps in a cell culture from another organ of the same species, or in a cell line from another animal species. In some instances, electron microscopic study of cell cultures in which the virus is grown may reveal the presence of characteristic virus particles. One of the most important methods employed in an attempt to detect a virus is that of bio-assay; under proper experimental conditions, a virus which either does or does not cause a cytopathic effect in tissue culture may still induce characteristic disease symptoms following inoculation into susceptible animals.

All these experimental tools directed at the detection of latent viruses have, however, considerable limitations; in spite of all attempts at its detection a virus may remain latent in cell cultures, and its presence may remain unrecognized. It may be very difficult to find the proper cell line in which such a latent virus may induce a cytopathic effect. The same applies to the bio-assay method; a virus may not necessarily be pathogenic for the animal species employed for the bio-assay tests, and yet it may remain fully pathogenic for another animal species not yet tested, and also for humans.

Early Isolation of Viruses from Normal Monkey Kidney Cells. Viral agents have long been isolated from normal monkey kidney cells. In 1933 Gay and Holden isolated the W virus; in 1934 Sabin and Wright recovered the B virus from a young physician who was bitten on the hand by an apparently healthy rhesus monkey in the laboratory and, as a result, developed an ascending myelitis which terminated fatally. Both viruses were related to the herpesvirus; it is generally believed that W and B viruses are identical. Six strains of virus B were isolated from pools of poliomyelitis virus which had been grown on rhesus monkey kidney cells; out of 650 pools examined, six were found contaminated with the B virus (Wood and Shimada, 1954).

In recent years it has been gradually recognized that monkey kidney cell cultures which have been so extensively employed in the preparation of vaccines contain a large number of latent indigenous viruses, most of which defy detection under the usual laboratory conditions.

How Many Latent Viruses in Normal Rhesus Monkey Kidney Cells? It has been noticed repeatedly that degenerative changes occur in the monkey kidney cells grown in tissue culture, even if they have not been seeded with any extraneous viruses. At first it was thought that such degenerative changes were due to a possible toxic effect of some substances present either in cells or in the media, or to some extraneous factors; however, it was realized later that these degenerative changes were frequently caused by cytopathic action of a latent virus or viruses present in such cells. At least some of these viruses were eventually isolated, and the cytopathic effect they cause was reproduced by transferring such viruses from one culture to another. Other viruses present in monkey kidney cells did not cause degenerative changes in cells in which they were naturally harbored, and their presence had to be determined by other methods; among such viruses many remained unidentified.

Rustigian and his colleagues (1955) reported that normal monkey kidney cells harbor several latent viruses which under certain conditions, such as serial passage in tissue culture, may become cytopathogenic. Among the isolated viruses one was designated as the "foamy virus" because of characteristic cytopathic changes it induces on passage in rhesus monkey kidney cells. During production and testing of poliomyelitis vaccines in which many thousands of monkey kidney cell cultures were prepared, many latent viruses were recovered from normal monkey kidney cells.

Hull and his colleagues (1956, 1957, 1958) at the Lilly Research Laboratories in Indianapolis, Indiana, described and classified a considerable number of simian viruses recovered from rhesus and cynomolgus monkey kidney cell cultures. At least some of these viruses caused distinct cytopathic changes on passage in rhesus monkey kidney cells. On the basis of cytopathic changes induced, Hull and his coworkers classified these simian viruses into 4 groups. Other viruses, also present in normal monkey kidney cells, could not be properly identified. There were so many of these agents that Hull and his colleagues suggested (1956) the designation of these viruses as "Simian Viruses" (S.V.) with added numbers, such as SV 1, SV 2, SV 3, etc. Viruses were also recovered from patas (*Erythrocebus patas*) monkey kidney cells by Hsiung and Gaylord (1961). At least 6 different viruses were recovered by Malherbe and Harwin (1957) from *Cercopithecus aethiops* monkey kidney cells; these viral agents caused characteristic cytopathic effect with formation of nuclear inclusion bodies and were designated as "Simian Agents" (S.A.) with added numbers, such as SA 1, SA 2, SA 3, etc.

The latent viruses of monkey kidney origin presented a real problem in the cell culture technique and in the preparation of vaccines. Such indigenous simian viruses contaminated, or in some instances even inhibited, the growth of the inoculated virus which was to be propagated in monkey kidney cultures. Most important of all, such viruses had to be excluded from any live virus vaccines prepared in monkey kidney cells and intended for use in man, since it was immediately realized that the long-term effect of human infection with such agents was unknown and had to be considered.

Isolation of the "Vacuolating Agent" from Normal Rhesus Monkey Kidney Cells

Most of the simian viruses derived from monkey kidney cell cultures could be detected in kidney cell cultures of the same monkey species, either in primary cultures or in further passages, because of the cytopathic effect they eventually exerted on the cells in which they were grown. This cytopathic effect could be recognized by a characteristic destruction of the cells in which such viruses were propagated, and frequently also by the formation of nuclear inclusion bodies. However, the question has often been raised whether there could also exist in monkey kidney cells latent viruses which may be present in such cells, but which could not be detected by current procedures because of their failure to induce degenerative changes in the cell cultures in which they were naturally harbored.

Formation of Characteristic Vacuoles in the Cytoplasm of Cercopithecus Monkey Kidney Cells Infected with the Newly Isolated Virus. Sweet and Hilleman at the Merck Institute for Therapeutic Research in West Point, Pennsylvania, reported in 1960 that in their tissue culture studies they observed what appeared to be a new simian virus of either rhesus or cynomolgus monkey kidney origin which did not cause any significant cytopathic changes in kidney cell cultures of the same species, and which was therefore undetectable when grown in its natural host cells; the same virus, however, caused very marked and distinctive cytopathic changes in kidney cell cultures of the African green monkey, *Cercopithecus aethiops*, i.e., grivet, obtained from Equatorial East Africa. Sweet and Hilleman referred to this newly isolated virus as the "*vacuolating agent*" because of the prominent development of vacuoles in the cytoplasm of cells infected with this virus. This cytopathic effect on the morphology of grivet kidney cells was very characteristic and appeared to be specific for this particular viral agent.

Formation of Intranuclear Inclusion Bodies. A few days prior to the development of the cytoplasmic vacuoles, formation of intranuclear inclusion bodies in cells infected with this virus could frequently be observed, particularly in patas monkey kidney cells in which the vacuolating agent was propagated (Hsiung and Gaylord, 1961, Gaylord and Hsiung, 1961), but also in vervet monkey kidney cells, and at least in some instances in rhesus kidney cells as well (Pay, 1961). However, intranuclear inclusions were also produced by other simian viruses, and an attempt to identify the vacuolating agent on the basis of nuclear chromatin alterations could have presented serious difficulties.

Sweet and Hilleman noted in their report (1960a,b) that even though the vacuolating agent, which they had just isolated, caused the characteristic cytopathic changes when grown in kidney cell cultures of the African green monkey, *Cercopithecus aethiops*, yet *Cercopithecus* monkey kidney cells were found to be remarkably free from contamination with this agent. In the course of more than 1000 lots of individual monkey kidney cell cultures examined, the presence of the vacuolating agent was detected only in one lot. On the other hand, this agent is very common, and essentially ubiquitous, in rhesus monkey kidney cell cultures, and is also a common contaminant of cynomolgus kidney cell cultures.

Albert B. Sabin Lloyd J. Old

John J. Trentin Wallace P. Rowe

Fig. 81

The "Vacuolating Agent" Is Designated "Simian Virus 40", i.e., "SV 40"

In an attempt to classify this virus, Hull suggested (Sweet and Hilleman, 1960a,b) that the vacuolating virus be included in group 4 of simian viruses causing cytopathic effect in tissue culture, and that it be designated "Simian Virus 40," i.e., "S.V. 40," or simply "SV 40" (Hull *et al.*, 1956, 1958. Hull and Minner, 1957. Hull, 1968).

In their initial report, Sweet and Hilleman concluded that the vacuolating agent "appears to be just one more of the troublesome simian agents to be screened and then eliminated from virus seed stocks and from live virus vaccines." They stressed at the same time, however, that the demonstration of the vacuolating agent as a common contaminant of rhesus and cynomolgus monkey kidney cells also raised the question of the possible presence of other indigenous and inapparent monkey kidney agents which might be detected with different methods of testing. The possible long-range pathogenic effects of either the SV 40 virus, or some other indigenous agents present in such cells, on their natural hosts, and particularly on humans, had to be considered.

> The vacuolating agent was first isolated and recognized in Dr. Hilleman's Virus and Tissue Culture Laboratories at the Merck Institute by L. McClelland in the course of safety testing of vaccines (Sweet and Hilleman, 1960).
>
> The presence of a previously undescribed latent simian virus in either rhesus (Pay, 1961) or patas (Hsiung and Gaylord, 1961) monkey kidney cells had been previously suspected. Sweet and Hilleman were the first, however, to identify, isolate, and pass this virus in different cell lines, to describe its physical and biological properties, and to recognize its ability to induce consistently the characteristic vacuolating degenerative changes in *Cercopithecus* monkey kidney cells, in contrast to its latency in rhesus monkey kidney cells, and thus to provide a test for the recognition and identification of this virus.

Isolation of the vacuolating agent (SV 40) by Sweet and Hilleman (1960a,b) marked a significant development in the study of indigenous simian viruses; such latent, and often difficult to detect, viruses had been frequently referred to as nothing more than troublesome agents interfering with normal production of commercial vaccines; however, the possible pathogenic potential of such viruses had long been considered. The newly isolated virus was latent in rhesus monkey kidney cell cultures, even though it multiplied in such cells to a considerable degree; the same virus, however, was consistently cytopathogenic for kidney cells of another, i.e., *Cercopithecus aethiops*, monkey species. This virus could now be readily identified, and it became immediately apparent that it is a very common contaminant of most of the rhesus monkey kidney cell cultures, and that most of the commercially available lots of polio and adenovirus vaccines employed for humans, including young children and in some instances also newborn human infants, contained traces of this virus. Unfortunately, this virus was found to have considerable resistance to heating and to formaldehyde, and for that reason not only live virus vaccines but also some of the formalin-inactivated vaccines were found to contain viable Simian Virus 40.

Accordingly, the question of the possible long-term pathogenic, and particularly oncogenic potential of this newly discovered and ubiquitous contaminant had to be seriously considered.

ONCOGENIC POTENCY OF "NORMAL" RHESUS KIDNEY CELL CULTURE EXTRACTS, DUE TO SV 40

The possible pathogenic potential of some of the latent viruses present in normal monkey kidney cell cultures employed extensively in recent years for the preparation of vaccines has long been considered by virologists and public health officials, and particularly by those concerned with the investigation of the safety of biological products prepared for use in humans.

Induction of Tumors in Hamsters with Normal Rhesus Kidney Cell Culture Extracts

The possibility that some of the latent viruses present in normal rhesus monkey kidney cell cultures may be potentially oncogenic was also considered. In order to test such a possibility, Dr. Bernice E. Eddy and her coworkers, Borman, Berkeley, and Young, at the Division of Biologics Standards of the National Institutes of Health in Bethesda, Md., carried out an experimental study (1958, 1961) in which tissue culture fluid collected from normal monkey kidney cell cultures was inoculated into newborn hamsters. Since the number of viruses present in monkey kidney cells was very large, it was impractical to test each virus individually; for that reason, two lots of rhesus monkey kidney cells taken from kidneys of 8 to 10 monkeys were incubated for 14 days, and the tissue culture fluids were then pooled and inoculated into 23 newborn hamsters.

In the initial experiment, 20 out of 23 injected hamsters developed tumors. The first tumor appeared after about 4 months, other tumors gradually later, some as late as 15 months after inoculation. The tumors, undifferentiated sarcomas, developed at the site of inoculation; they increased rapidly in size, some of them eventually becoming as large as their hosts (Eddy *et al.*, 1961).

The experiment was repeated. Fluid collected from normal monkey kidney cell cultures was inoculated into 154 newborn hamsters, and as a result, 109 of them (70 percent) developed tumors. The extract could be passed through a bacterial Selas 03 filter candle and still retained its oncogenic potential; out of 14 newborn hamsters inoculated with such a filtered extract, 13 developed tumors. The earliest tumor was detected after 3 months, but most of the tumors developed after 9 months. All tumors developed at the site of inoculation as single, occasionally multiple, subcutaneous sarcomas. Some hamsters had tumors in the lungs or kidneys as well.

The tumors could be transplanted without difficulty by cell-graft to newborn hamsters; they developed in the inoculated animals after two to three weeks and grew progressively.

An Unidentified Oncogenic "Substance" in Normal Monkey Kidney Cells Posing a Serious Safety Problem. The observation that extracts prepared from normal rhesus kidney cell cultures induced malignant tumors following inoculation into newborn hamsters posed a serious problem, since such cultures were used routinely to propagate poliovirus and adenoviruses for the production of vaccines. It was imperative to determine the source of the oncogenic material in normal monkey kidney cell cultures. However, in the initial experiments carried out by Eddy and her coworkers (1961), no virus could be recovered from the tumors which had been induced in hamsters with normal monkey cell extracts. Several attempts were made to recover a virus; extracts made from the induced tumors were inoculated into newborn hamsters, but no tumors developed in the injected animals; in another experiment tumor extracts were inoculated into several cell lines, such as mouse embryo, rhesus monkey, and vervet monkey kidney cells, but no definite evidence of virus growth could be obtained in tissue culture. Failure to recover a virus from the induced tumors led Dr. Eddy and her coworkers (1961) to designate the tumor-inducing material present in normal rhesus monkey kidney cells as a "substance."

Tumors Induced in Hamsters with Normal Monkey Kidney Cell Extracts Were Not Caused by Polyoma Virus. It was necessary to exclude the possibility that the inoculated extracts contained the polyoma virus, since it is known that the parotid tumor, i.e., polyoma, virus is also capable of inducing subcutaneous sarcomas following inoculation into newborn hamsters. However, although there were similarities in microscopic morphology between the tumors induced in hamsters by monkey kidney cell extracts and those induced by the polyoma virus, there was a striking difference in the distribution of the tumors. The

monkey kidney cell extracts consistently induced subcutaneous sarcomas, and in a few instances also tumors in the lungs and kidneys. On the other hand, if a sufficient dose of polyoma virus was inoculated into newborn hamsters, not only subcutaneous sarcomas were induced, but also tumors or hemangiomas in the heart muscle, in the liver, stomach, intestines, and other internal organs. No such tumors, and particularly no hemangiomas, were induced with the monkey kidney cell extracts.

The polyoma virus causes hemagglutination of red blood cells which can be inhibited by a specific immune serum collected from animals infected with this virus. However, extracts of either tumors or organs of hamsters which developed sarcomas as a result of inoculation of normal monkey kidney cell extracts did not agglutinate erythrocytes; furthermore, the serum from such animals did not inhibit the agglutinating ability of the polyoma virus.

It was quite apparent, therefore, that the oncogenic potency of normal rhesus monkey kidney cell extracts for hamsters was not due to the presence in such extracts of the polyoma virus (Eddy *et al.*, 1961).

Identification of the Tumor-inducing "Substance" Present in Normal Rhesus Monkey Kidney Cells as Simian Virus 40

The striking oncogenic potential of the vacuolating agent was discovered simultaneously in two laboratories. Eddy and her coworkers, who only one year earlier (1961) had observed that normal rhesus monkey kidney cell extracts induced malignant tumors following inoculation into newborn hamsters, were now able to identify the oncogenic "substance" present in such extracts as the vacuolating agent, i.e., the SV 40 virus. After initial unsuccessful attempts (1961), Eddy and her coworkers now succeeded (1962) in recovering, by intermediate passage through tissue culture, the Simian Virus 40 from tumors which they had induced in hamsters with normal rhesus monkey kidney cell extracts. Furthermore, they reported at the same time that an anti-SV 40 immune serum neutralized the oncogenic potency of such extracts (Eddy *et al.*, May 1962a).

Induction of Tumors in Hamsters with Cultures of Simian Virus 40. At about the same time, Girardi and his coworkers reported on April 13, 1962, at the Annual Meeting of the American Association for Cancer Research in Atlantic City, N.J., that inoculation of SV 40 virus cultures into newborn hamsters resulted in the development of subcutaneous progressively growing malignant sarcomas in most of the inoculated animals. (Girardi *et al.*, 1962a,b. Girardi and Hilleman, 1964).

It was therefore recognized independently in two different laboratories that the SV 40 virus has a considerable oncogenic potential for hamsters. The possible oncogenic potential of this common contaminant of normal monkey kidney cell cultures for other animal species, including humans, raised a serious problem concerning the safety of many lots of vaccines prepared in monkey kidney cells for use in human patients.

The induction of tumors with the Simian Virus 40 in hamsters and in other laboratory animals will be discussed in more detail in a subsequent section of this chapter.

Propagation of Simian Virus 40 in Cell Cultures

Permissive and Nonpermissive Cells. Cells that support the replication of SV 40 and as a result are destroyed by lysis, are called *permissive cells;* on the other hand, those cells that do not support replication of the virus but may be transformed following infection with SV 40 are called *nonpermissive* cells. The permissive cells are found among monkey cells, i.e., cells of the natural host species for SV 40.

When *nonpermissive cells* are infected with SV 40, most of them survive, since they do not support virus replication, but they may be transformed. There also exist *semipermissive cells;* in such cell populations, some cells support virus replication and are destroyed by lysis, whereas other cells survive the infection, but may become permanently transformed. Human cells, and also rabbit cells, rat cells, or hamster cells, represent examples of semipermissive cells for SV 40.

Lytic Infection in Permissive Cells. Replication of SV 40. When the Simian Virus 40 infects a susceptible cell, it is adsorbed at the cell surface, then enters the cell and sheds its coat; the viral genome, liberated from its capsid, is then replicated in the susceptible cell within 12 hours after infection. New virus particles are then formed; about 36 hours after infection, the cells begin to die, liberating the newly formed virus particles. This course of events is very similar to that which follows the infection of susceptible cells by the polyoma virus.

Cytopathogenic Effect (CPE). In some of the susceptible cells which allow at least a partial virus replication, the destructive effect of the virus on the cells (cytopathogenic effect, i.e., CPE) can be followed on inspection of cell cultures and is of diagnostic value.

The morphologic changes that occur in virus-infected cells tend to be characteristic of a specific virus-cell system. CPE does not usually offer a technique for virus identification but it does provide a foundation for a preliminary grouping on a pathological basis. The cytopathogenic effect of the Simian Virus 40 on *Cercopithecus aethiops* kidney cells, described hereafter, suggested the initial characterization of this agent as "vacuolating agent."

SV 40—A Common Contaminant of Monkey Kidney Cell Cultures. The Simian Virus 40 is a common contaminant of normal Asian rhesus monkey (*Macaca mulatta*), and less frequently also of cynomolgus monkey kidney cell cultures. SV 40 is, or rather was, essentially ubiquitous in various virus seed stocks and vaccines, suggesting a high infection rate with this virus among normal monkey kidney cell cultures.

> Since the discovery of the oncogenic potency of Simian Virus 40, described on subsequent pages of this chapter, considerable efforts have been made to eliminate this virus, as well as any other potentially pathogenic agents, from cell cultures employed for the preparation of vaccines.

Propagation of SV 40 in Monkey Kidney Cells. SV 40 can readily be propagated in rhesus and cynomolgus monkey kidney cells and attains a high titer in such cultures without inducing any of the destructive changes in the morphology of infected cells which would indicate the presence of the virus. The virus can also be propagated in grivet, i.e., *Cercopithecus,* monkey kidney cells; however, in the grivet kidney cells the virus induces a characteristic cytopathic effect. In addition to being cytopathogenic for the grivet monkey, the virus was also found to be cytopathogenic for the vervet monkey kidney cells.

> There are now some 20 recognized subspecies of the *Cercopithecus* group of monkeys, but the differences between many of them are very slight. They are spread around Africa from Senegambia to Abyssinia (Ethiopia) and thence south to the Cape. They are known as *vervets* in South Africa, as *grivets* in northern areas, and as *green monkeys* in West Africa (Sanderson, 1957. Miller, 1961).

The SV 40 was also cytopathogenic for the, closely to the grivet related, patas (*Erythrocebus patas*) monkey kidney cells, and for rhesus monkey testicle cells; however, these cells were about 30 to 300 times less sensitive than grivet kidney cells to the cytopathogenic action of the Simian Virus 40.

Cytopathic Changes Induced with SV 40 in Cercopithecus Aethiops Kidney Cells. The cytopathic changes induced with SV 40 in green monkey, i.e., *Cercopithecus aethiops,* kidney cells are very characteristic for this particular virus and quite different from those induced by other monkey kidney cell viruses previously described. Following inoculation of the grivet kidney cell cultures with a sufficient dose of the virus, the first characteristic changes in the cell morphology begin to appear 3 to 4 days later; some of the cells appear rounded or shrunken, and a few may show a beginning vacuolation of the cytoplasm. Soon typical changes develop, consisting predominantly of ballooning of cells and intense vacuolation of the cytoplasm of such cells. These vacuoles are highly characteristic, particularly in wet preparations; in hematoxylin and eosin stained material they appear as "holes" with intensely stained boundaries. Five to 10 days after inoculation of the virus the cells aggregate together and detach from the glass. These cytopathic changes are very specific and can be recognized at once. Intense vacuolation of the infected cells led to the initial designation of this virus as the "vacuolating agent" later changed to "Simian Virus 40" (SV 40).

Propagation of Simian Virus 40 in Human Cells in Tissue Culture and Induction of Cell Transformation

Several successful attempts have been made to propagate SV 40 in human cells in tissue culture (Sweet and Hilleman, 1960. Hsiung and Gaylord, 1961. Eddy *et al.*, 1962). At the Children's Hospital Medical Center in Boston, Shein and Enders (1962a,b) observed that Simian Virus 40 could be propagated in human cell lines, inducing no cytopathic effect on the first passage; after subsequent passages, when it was transferred into human kidney cell cultures, proliferating foci were induced which became progressively large. Eventually, epithelioid transformation of primary cultures of human renal cells was observed in such infected cultures.

The fact that human cell cultures could be infected with SV 40, and that such cultures could carry the virus in continuous passage was of considerable interest. The viral infectivity attained maximum titers by the 25th to 41st day after inoculation and remained constant during several weeks (Shein *et al.*, 1962). Most striking, however, was the appearance of epithelioid cell forms, with abnormal growth pattern and chromosomal aberrations.

In a similar study, Koprowski and his colleagues (1962), at the Wistar Institute in Philadelphia, inoculated organ cultures, prepared

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FIG. 82. SV 40 VIRUS PARTICLES

(A). Section of *Cercopithecus* kidney cell 10 days after infection with SV 40 virus. Intranuclear virus particles. Magnification 30,000 ×. (B). Section of *Cercopithecus* kidney cell 10 days after infection with SV 40 virus. Intranuclear crystalline array of small uniform virus particles. The particles have no external membrane, but present a clear zone on their periphery, which may correspond to the capsid. Magnification 70,000 ×. Electron micrographs prepared by N. Granboulan, Institut de Recherches Scientifiques sur le Cancer, Villejuif (Seine), France.

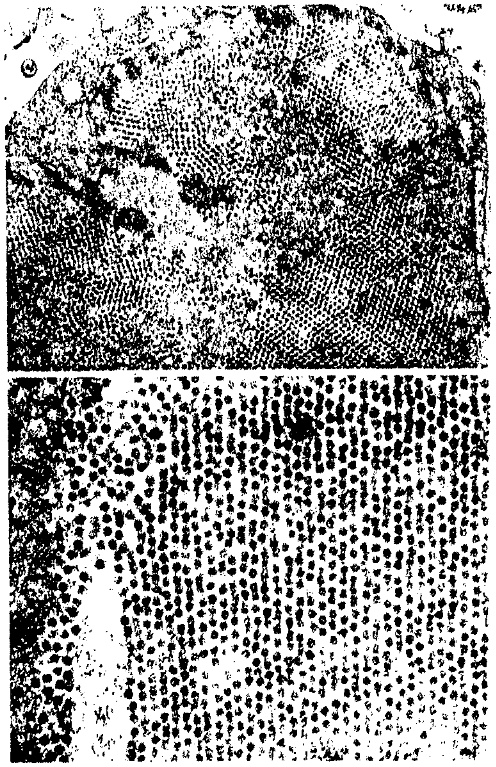

FIG. 82. SV 40 VIRUS PARTICLES

from human skin and buccal mucosa, with SV 40. A transformation could be observed in the infected cell cultures 8 to 14 weeks after exposure to the virus. This transformation was characterized by the appearance of colonies of morphologically abnormal cells of an irregular epithelial-like growth pattern; these cells eventually spread and overgrew the normal cell population. Distinct chromosomal abnormalities accompanied this cell transformation.

The alterations in the morphology of human cells grown in tissue culture, as well as chromosomal abnormalities observed after introduction of SV 40, raise the serious problem of a possible long-term oncogenic potential of this virus in humans.

Enhancement of Adenovirus Growth in Rhesus Monkey Kidney Cells by Presence of SV 40. The demonstration of oncogenic potency of SV 40 led to numerous efforts directed at removing any traces of the vacuolating agent from pools of poliomyelitis virus or adenoviruses which were to be used for the preparation of vaccines. Among the methods employed, treatment with appropriate immune serum directed against SV 40 was successfully used. However, in the course of these studies it was found that this treatment could not be applied to adenoviruses. It appeared that cultures of certain types of adenoviruses containing a slight contamination with SV 40 could not readily be freed from SV 40, without at the same time impairing the propagation of the adenoviruses grown in such cell cultures. This applied particularly to type 4 adenovirus. It appeared that adenovirus type 4 did not propagate in grivet monkey kidney cells in the absence of SV 40. Very small amount of residual infection with SV 40 was required, but it appeared to be necessary for adenovirus replication (Rowe and Baum, 1964, 1965. Huebner *et al.*, 1964. Rowe *et al.*, 1965).

Similar observations were later made with adenovirus types 5 and 12 (Rabson *et al.*, 1964) showing that an enhancement of growth of adenovirus types 5 and 12 in African green monkey kidney cells was noticed if such cell cultures were preinfected with SV 40. The presence of SV 40 antigen was demonstrated in cell cultures infected with type 7 adenovirus that had been contaminated with, but subsequently freed from, detectable SV 40 (Rapp *et al.*, 1964. Huebner *et al.*, 1964. Rowe *et al.*, 1965). It is possible that there may occur some linkage, or other association, of SV 40 with adenovirus type 7, whether by genetic mixing or perhaps by hybridization.

Adeno-SV 40 Virus Hybrids. The problem of safety of vaccines prepared on monkey kidney cells became more complex when it was discovered that some of the SV 40 virus particles present in a latent form in rhesus monkey kidney cells could become incorporated into type 7 adenoviruses, resulting in the formation of "virus hybrids" which possessed the oncogenic potential of the Simian Virus 40

(Huebner *et al.*, 1964. Rowe, 1965. Rowe and Baum, 1965. Black and Todaro, 1965. Lewis, 1973. For additional information and pertinent references, see Tooze, 1973).

Electron Microscopic Studies

Tournier, Granboulan, and Bernhard (1961) examined in the electron microscope ultrathin sections of *Cercopithecus aethiops* kidney cells infected with SV 40. Beginning with the second day after virus inoculation, and more extensively a day or two later, characteristic changes could be observed in the nuclei of the infected cells, consisting of margination and development of irregularly-shaped dense areas in the chromatin with a simultaneous striking appearance of innumerable small spherical virus particles; these particles were homogenous, and had no external membranes. The average diameter of individual particles was 33 nm; the center-to-center distance of packed particles within the crystalline arrays was 40 to 42 nm. The regularity of the size of the individual particles was striking (Granboulan *et al.*, 1963).

At the same time characteristic lesions could be observed in the nucleoli, consisting of development of very dense areas containing granules and also filaments. With each advancing day the number of virus particles in the cell nuclei increased, until they formed crystalline arrays of packed virus particles which practically filled out the entire nucleus. Between the fifth and tenth day after infection, most of the cell nuclei were found to be filled with virus particles. At this phase, virus particles appeared also in the cytoplasm, and the cells began to disintegrate.

In the cytoplasm the number, and particularly the size, of the vacuoles increased considerably following infection with the virus; this observation corresponded closely to the cytopathic effect of the virus on the cells described in the studies with the light microscope. Virus particles could be found in the cytoplasm in general after 6 days following infection, and in some cases earlier (Granboulan *et al.*, 1963).

Gaylord and Hsiung (1961) studied Simian Virus 40 propagated on *Erythrocebus patas* kidney cells. They observed formation in the infected cells of nuclear inclusion bodies which contained virus particles. In advanced phases the nuclei were almost entirely filled with virus particles. The particles were spherical, homogenous, and about 30 nm in diameter.

It is of interest that SV 40 virus particles could be detected with the electron microscope without difficulty in infected monkey kidney cells in tissue culture, but not in tumors induced in hamsters with

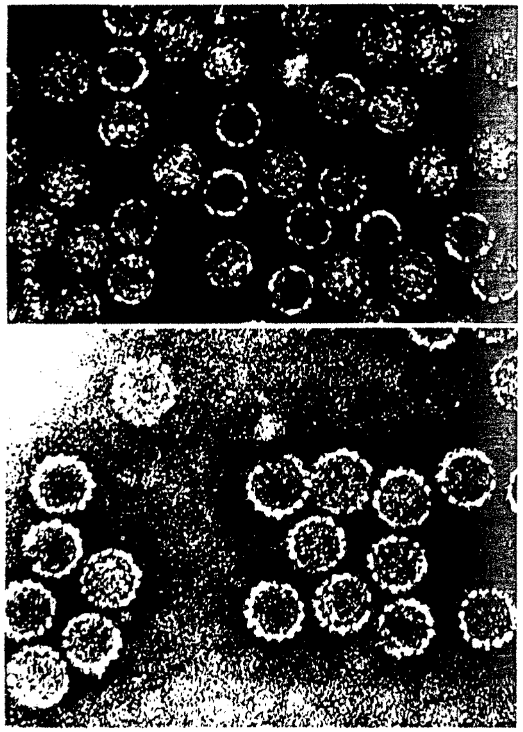

FIG. 83. ELECTRON MICROGRAPHS OF SV 40 VIRUS PARTICLES IN NEGATIVELY STAINED PREPARATIONS

the same virus. This interesting observation will be discussed in more detail on subsequent pages of this chapter.

Antigenic Properties of Simian Virus 40

Simian Virus 40 is strongly antigenic. A specific immune serum could be obtained from rabbits that had been immunized with several injections of this virus. The antibodies could be detected either by direct virus neutralization tests, or by complement fixation. In the virus neutralization tests the oncogenic potency of tissue culture fluid containing Simian Virus 40 could be completely neutralized by a specific anti-SV 40 immune rabbit serum. In a more rapid test, the cytopathic effect of the virus, i.e., the formation of characteristic vacuoles in the cytoplasm of *Cercopithecus* monkey kidney cells in tissue culture, could be inhibited following neutralization of the virus *in vitro* by a specific immune serum.

Antigenically, the SV 40 is distinct from any other known viruses; no antigenic variants of SV 40 have yet been described. All strains of Simian Virus 40 studied until now have been of the same single immunologic group.

Specific antibodies directed against SV 40 could be detected in monkeys carrying this virus as a latent contaminant, and also in hosts of different species, including monkeys and humans, which had received this virus by inoculation.

Some Properties of the Simian Virus 40

Resistance to Heat, Ether, and Formaldehyde. The Simian Virus 40 is remarkably resistant to heat. There is only a moderate loss of viral activity after heating the virus to 60°C for 30 minutes; in certain experiments the presence of some infectivity could still be demonstrated after heating the virus to 70°C for 30 minutes.

The virus is resistant to treatment for 18 hours with ethyl ether. It is of considerable practical importance that SV 40 is also relatively resistant to formaldehyde; it is not inactivated by a concentration of

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FIG. 83. ELECTRON MICROGRAPHS OF SV 40 VIRUS PARTICLES IN NEGATIVELY STAINED PREPARATIONS

(A). SV 40 virus particles in negatively stained preparation treated with phosphotungstic acid. Magnification 280,000 ×. (B). SV 40 virus particles in negatively stained preparation treated with phosphotungstic acid. Magnification 400,000 ×. Electron micrographs prepared (A) by C. Vasquez and (B) by N. Granboulan, W. Bernhard and P. Tournier, Institut de Recherches Scientifiques sur le Cancer, Villejuif (Seine), France.

formaldehyde which is sufficiently strong to inactivate polio or adenoviruses; viable SV 40 could be recovered from certain lots of formalin-inactivated adenovirus, or from Salk polio vaccines. Most of the SV 40 present in tissue culture fluid could be inactivated with 1 : 4000 formaldehyde at 37°C, but less rapidly than the poliomyelitis virus.

No Hemagglutinating Activity. Culture fluid containing the SV 40 does not cause hemagglutination of guinea pig, chicken, or human "O" erythrocytes, at 4°C, or at 25°C. Furthermore, the virus is not adsorbed by red blood cells.

THE CHEMICAL COMPOSITION AND PHYSICAL STRUCTURE OF SV 40

Since the early studies which demonstrated that the virus-containing inclusions in the cytoplasm were Feulgen-positive (Gaylord and Hsiung, 1961), it was realized that SV 40 is a DNA virus. Isolation of infectious DNA from the Simian Virus 40 was also reported in the early studies (Boiron *et al.*, 1962. Gerber, 1962). The viral core structure consisting of a double-stranded DNA was soon recognized (Mayor *et al.*, 1963).

SV 40 is a very small virus particle, about 45 nm in diameter, similar in its structure, chemical composition, and in its molecular weight to the polyoma virus reviewed in the preceding chapter (Weil, 1978). About 88 percent of each SV 40 or polyoma virus particle is protein; the remaining 12 percent is DNA. There are no detectable lipids or carbohydrates.

The absence of any detectable lipids explains the fact that SV 40 as well as polyoma and other viruses of the papova group are resistant to liquid solvents, such as ethyl ether. The major capsid proteins of SV 40 and the polyoma virus particles account for about 70 to 80 percent of the total protein.

The genomes of the SV 40, of the polyoma virus, and of related papilloma viruses are double-stranded, circular DNA molecules; they are infective, and they carry the entire genetic information. In its base composition, however, the viral DNA is characteristic for each virus.

The Fine Structure of SV 40. Melnick (1962), Bernhard and his coworkers (1962), and also Horne (1963a,b) studied SV 40 particles by the negative staining method and found that these particles are made up of 42 capsomers in a 5:3:2 (icosahedral) pattern, as is the polyoma virus. Subsequent studies revealed, however, that the capsids of these viruses consist of 72 capsomers (subunits) arranged in icosahedral

structure (Klug, 1965. Klug and Finch, 1965, 1968. Mattern *et al.*, 1967. Koch *et al.*, 1967). The diameter of negatively stained SV 40 and of the polyoma virus particles is about 45 nm (Wildy *et al.*, 1960).

For a detailed description of the physical and chemical composition of the SV 40 and polyoma virus particles, of the structure of the viral DNA, and for pertinent references, the reader is referred to *The Molecular Biology of Tumour Viruses* by J. Tooze (1973) and to a more recent comprehensive review by Weil (1978).

The Papova Virus Group

The SV 40, polyoma, and papilloma viruses belong to the *papova* virus group described on page 872 in the following chapter. Members of this group have a DNA core enclosed in a protein capsid with 72 capsomers. Among other characteristic features of this group is the absence of lipids, thermal resistance, multiplication within the cell nucleus, and their oncogenic potential (Melnick, 1962).

Routes of Natural Transmission of Simian Virus 40 in Its Natural Carrier

The spread of Simian Virus 40 under natural life conditions in its simian hosts has not yet been sufficiently clarified, and the information thus far available is limited. The virus does not seem to induce definite symptoms of disease in its rhesus carrier, or in other related monkey species, and the presence of infection can be determined only on the basis of development of specific humoral antibodies in the infected animals or by isolation of the virus from either nasopharyngeal swabs or from feces of such hosts. Information thus far accumulated suggests that the virus can be transmitted by respiratory route, by intimate contact of susceptible hosts exposed to animals which excrete the virus, and possibly to some limited extent also by the oral route. Contact infection did occur in green monkeys caged with virus-carrying animals (Meyer *et al.*, 1962). Experimentally, non-immune rhesus monkeys are readily infected by the oral, intranasal and subcutaneous routes, and viremia and viruria occur in infected animals (Shah *et al.*, 1969). Virus may persist in the kidneys in a latent form. Infection is apparently harmless for these natural hosts. Although the exact manner of transmission is not known, the most likely possibility is that the virus, excreted in the urine, is transmitted to susceptible monkeys by the respiratory or oral routes. Simian Virus 40, like the polyoma virus, appears to induce urinary tract infections which lead to elimination of the virus in the urine.

Induction of Tumors in Hamsters Following Inoculation of Simian Virus 40

Following inoculation of Simian Virus 40 into newborn Syrian hamsters, most of the inoculated animals developed either single or multiple tumors at the site of inoculation after a latency varying from 2½ to 3 months. In addition, some animals developed similar tumors also in lungs and kidneys. If a sufficient dose of the virus was inoculated, practically all animals developed tumors. The induced subcutaneous tumors consisted of hard masses which infiltrated the skin and subcutaneous tissues, and invaded the adjacent muscles. The tumors grew progressively, and never regressed; in some instances they eventually became larger than the rest of the animal.

Influence of Age at Inoculation. Newborn and less than 2-day-old suckling hamsters were uniformly susceptible to the oncogenic potency of SV 40; over 95 percent of such animals developed tumors following inoculation of a sufficient dose of virus. The susceptibility of hamsters decreased rapidly with increasing age. When hamsters were inoculated at 7 to 8 days of age, about 60 percent still developed tumors, but the latency was prolonged. The animals remained relatively susceptible to inoculation up to about 3 weeks of age, but the incidence of induced tumors was substantially lower in the older group, and the latency considerably prolonged. Girardi and his coworkers (1963) observed a few tumors in hamsters that had been inoculated at one month of age; tumors appeared in such animals after a latency of about one year. No tumors could be induced when older animals were inoculated. Eddy and her coworkers (1962b) did not observe development of tumors when hamsters more than three weeks old were inoculated.

> The susceptibility of the newborn hamsters to the oncogenic potency of SV 40, and the rapid development of relative resistance with increasing age, bore a striking resemblance to earlier observations recorded in the study of mouse leukemia (Gross, 1951).

Routes of Inoculation, and Dose of the Injected Virus. Several routes of inoculation were tested in the early experiments, such as subcutaneous, intraperitoneal, intrathoracic, and intracerebral; tumors could be induced by any of these routes, but the subcutaneous route of virus inoculation was found to be most suitable for the induction of tumors (Eddy *et al.*, 1961, 1962b). As a rule, tumors were induced at the site of inoculation; following intraperitoneal or intrathoracic inoculation, tumors could be induced in the peritoneal or in the chest cavities, but frequently in such animals subcutaneous tumors developed also at the site where the virus was introduced through the skin puncture. In some experiments, only subcutaneous tumors developed even following intraperitoneal or intrathoracic inoculation (Girardi *et al.*, 1962b). All these tumors, including neoplasms developing in the abdominal and chest cavities, had a similar microscopic morphology, i.e., that of undifferentiated sarcomas.

Intracerebral inoculation of SV 40 into newborn hamsters resulted in the development of ependymomas (Kirschstein and Gerber, 1962. Eddy, 1962).

There was a correlation between the injected doses and the incidence and time of development of tumors. A higher incidence of tumors developing after a shorter latency was observed after inoculation of higher doses of the virus.

Morphology of the Induced Tumors. All tumors developing in hamsters following either subcutaneous, intraperitoneal, or intrathoracic inoculation of the virus were sarcomas. These tumors grew progressively, infiltrating the adjacent tissues. Microscopic examination revealed that these tumors were undifferentiated sarcomas, frequently containing multinucleated giant cells. Parts of some of the subcutaneous tumors had the morphology of fibrosarcomas. The ependymomas developing in the brain as a result of intracerebral inoculation of SV 40 were gliomas, brain tumors, arising from the ependymal lining of the ventricles, and consisting of papillary masses of cuboidal cells with darkly stained nuclei and scant cytoplasm.

Transplantation of the Induced Tumors. The induced tumors could be transplanted without difficulty from one hamster to another either in newborn or young adult animals. Following transplantation by cell-graft, tumors developed in the inoculated animals after a latency of 2 to 3 weeks; the transplanted tumors grew progressively.

Difficulty in Recovering Virus from the Induced Tumors

No virus could be recovered from the tumors in sufficient quantity in order to pass the virus directly from one animal to another. However, the virus could be recovered by intermediary passage through tissue culture. Following inoculation of *Cercopithecus* kidney cell cultures with tumor extracts, a characteristic cytopathic effect consisting of the formation of vacuoles in the cytoplasm of infected cells could be detected in most of the infected cultures; frequently, however, this occurred only after a prolonged incubation, or after a preliminary blind passage made in order to increase the titer of the isolated virus. More virus was present in large than in small tumors. However, often many difficulties were encountered in recovering the virus from the induced tumors (Girardi *et al.*, 1962. Eddy, 1962. Sabin and Koch, 1963. Ashkenazi and Melnick, 1963). In general, only very *small quantities of virus* could be recovered from the induced primary or transplanted tumors, and frequently no detectable virus at all could be recovered from some of the tumors.

Failure to Detect Virus Particles on Electron Microscopic Examination of Tumor Cells. Electron microscopic examination did not reveal the presence of virus particles in the induced tumors, in contrast to the commonly accomplished demonstration of the presence of such particles in infected tissue culture cells.

The failure to detect virus particles in the induced tumors on electron microscopic examination recalled similar difficulties encountered in experiments dealing with tumors induced by either adenoviruses or by the polyoma virus. In these experiments, discussed in separate chapters of this monograph, there were difficulties in recovering the virus directly from tumors that had been induced by either of these viruses; furthermore, no virus particles could be detected on electron microscopic examination of such tumors. As in the case of experiments dealing with SV 40, these difficulties contrasted sharply with the successful isolation of adenoviruses or polyoma virus from the induced tumors following an intermediary tissue culture passage, and also with the ready detection of characteristic virus particles on electron microscopic examination of infected cells in which either of these two viruses had been grown in tissue culture.

Induction of Brain Tumors (Ependymomas) in Mastomys with Simian Virus 40

Rabson and his coworkers (1962) at the Department of Pathology of the National Cancer Institute in Bethesda, Md., reported that subcutaneous inoculation of newborn *Mastomys* (*Rattus Mastomys natalensis*), an African rodent, with SV 40 resulted in the development of brain tumors having histopathologic characteristics of papillary ependymomas. These tumors have been found in 8 out of 10 animals that survived the inoculation trauma. The development of tumors became apparent after periods varying from 111 to 225 days.

Rattus (*Mastomys*) *natalensis* is an African rodent, intermediate in size between the rat and mouse; it has been employed in cancer research in recent years because of the high incidence of spontaneous adenocarcinoma of the stomach observed in these animals (Oettlé *et al.*, 1959) and because of the susceptibility of *Mastomys* to tumor induction by the polyoma virus (Rabson *et al.*, 1960) and the adenoviruses (Rabson *et al.*, 1964).

These experiments were of considerable interest; they demonstrated that SV 40 could induce tumors not only in hamsters, but also in another animal species; moreover, it was of particular interest that subcutaneous inoculation of an oncogenic virus could induce

brain tumors without the development of either subcutaneous or visceral neoplasms resulting from such inoculation.

Papillary ependymomas could also be induced in hamsters following direct intracerebral inoculation of SV 40 into newborn hamsters (Kirschstein and Gerber, 1962. Eddy, 1962).

Induction of Leukemia, Lymphosarcomas, and Osteosarcomas in Hamsters by Intravenous Inoculation by SV 40

SV 40 induced lymphocytic leukemia, lymphosarcomas, reticulum-cell sarcomas, and osteosarcomas following intravenous inoculation of relatively large doses of the virus by intravenous route into 3-week- or 1- to 2-month-old Syrian golden hamsters (Diamandopoulos, 1972, 1973). Only seldom did either leukemia or tumors develop when 3- to 6-month-old hamsters were inoculated. No tumors or leukemia were induced when animals more than 6 months old were inoculated. Only those animals which received a sufficient dose of the virus developed tumors or leukemia. The younger animals developed tumors after a relatively short latency; when older animals were inoculated, tumors appeared very late or did not appear at all. Leukemia, lymphosarcomas, reticulum cell sarcomas, and osteosarcomas developed only in those hamsters that received the virus by intravenous route, whereas anaplastic and spindle cell sarcomas developed in hamsters following subcutaneous or intramuscular inoculations (Diamandopoulos and McLane, 1975). Evidence for the etiologic role of the SV 40 virus was obtained by neutralizing the oncogenic effect of the virus by an anti-SV 40 immune serum and also by demonstrating the presence of SV 40 T (tumor) antigens in the cells of all tumors tested.

The fact that SV 40 may induce lymphocytic leukemia, lymphosarcomas, or osteogenic sarcomas was unexpected and rather surprising. Particularly interesting was the fact that leukemia and malignant lymphomas could be induced with a DNA virus, other than a herpesvirus.

Under certain experimental conditions lymphocytic leukemia induced with the Simian Virus 40 could be serially passaged by cell-graft in syngeneic 3-week-old hamsters (Diamandopoulos, 1978); however, no recovery of Simian Virus 40 from the leukemic cells was reported. In this respect, the SV 40-induced leukemia was not different from the sarcomas induced in hamsters following subcutaneous or intramuscular inoculation of the same virus in earlier experiments (Eddy, 1962. Girardi *et al.*, 1963).

It is anticipated that these interesting, relatively recent, observations will be confirmed by other investigators. Up to the time of this writing, however, no confirmation has yet been reported from other laboratories (personal communication to the author from Dr. G. T. Diamandopoulos, April, 1981.)

Effect of SV 40 Infection on Its Natural Carrier Host

Simian Virus 40 is a natural infection of a few species of Asiatic macaques, in particular the rhesus (*M. mulatta*). On the other hand the cynomolgus monkey of East Asia (*M. fascicularis*) and the African green monkey (*Cercopithecus aethiops*) seem to be free from SV 40 (Hull, 1968. Meyer, *et al.*, 1962. Shah and Nathanson, 1976).

Infection is apparently completely harmless for the natural hosts. The virus is excreted in the urine and is presumably transmitted to susceptible monkeys by the respiratory and oral routes. Many very young rhesus are free of SV 40 antibodies when they are captured, but infection subsequently promptly occurs, following contact with virus-carrying animals. Experimental infection with SV 40 of the African green (*Cercopithecus aethiops*) monkeys (Meyer *et al.*, 1962), or inoculation of Simian Virus 40 into rhesus monkeys apparently free from active SV 40 infection (Shah *et al.*, 1969) resulted in viremia, but no apparent illness.

Ashkenazi and Melnick (1962), at Baylor University in Houston, inoculated Simian Virus 40 into seven baboons (*Papio doguera*) about 6 months old, and 12 green monkeys (*Cercopithecus aethiops*); the latter included eight young one-year-old animals and four infant seven-day-old monkeys. Both the green monkeys and the baboons are usually free from the SV 40 virus unless they are placed in contact with the infected Asian animals (Sweet and Hilleman, 1960. Meyer *et al.*, 1962. Ashkenazi and Melnick, 1962). The inoculations were made mostly by subcutaneous route; some of the animals received the virus intracutaneously or intracranially, and in a few instances by intranasal instillation or by oral route. No symptoms of illness were induced in any of the inoculated animals, but there was evidence that the virus persisted and multiplied more abundantly in the green monkeys than in the baboons. SV 40 was found to be excreted in the urine collected from green monkeys during the first four weeks after infection. Virus was also recovered from the urine of all four baboons inoculated parenterally, but not from three baboons infected by intranasal or oral administration of virus. Later, in the course of infection, neutralizing antibodies appeared in blood and also in urine of these animals.

In another study, experimental infection of *Cercopithecus* monkeys with SV 40 by intranasal instillation resulted in viremia; again no illness resulted from the administration of the virus. However, a transient presence of the virus in the pharyngeal secretions and in the stools of the inoculated monkeys could be revealed in such animals during the first two weeks after inoculation; later on neutralizing antibodies appeared in the serum of the inoculated monkeys reaching a maximum titer by the seventh week (Meyer *et al.*, 1962). The virus was transmitted by contact infection to another uninoculated *Cercopithecus* monkey which was placed in the same cage; this was evident by the appearance of specific antibodies in that animal.

What Is the Effect of Simian Virus 40 on Humans?

No disease symptoms were apparently induced in humans following infection with this virus; however, observations thus far reported suggest that SV 40 can multiply in human hosts to some degree and that at least a transient inapparent infection with this virus can be induced. The fact that a low-grade latent infection with SV 40 could be established in humans following inoculation of this virus became evident after it was demonstrated that specific antibodies directed against SV 40 appeared in subjects which had received this virus inadvertently in small quantities during parenteral administration of poliomyelitis or adenovirus vaccines. Live SV 40 in traces, or sometimes in moderate quantities, was present as a contaminant of vaccines prepared from virus pools grown in rhesus and cynomolgus kidney cell cultures (Sweet and Hilleman, 1960. Gerber *et al.*, 1961. Goffe *et al.*, 1961. Magrath *et al.*, 1961. Melnick, 1962). The monkey kidney cell cultures employed for the propagation of virus pools frequently contained the Simian Virus 40 and for that reason vaccines prepared from such cultures also contained this virus. Not only live polio vaccines, but formalin-inactivated vaccines frequently contained viable SV 40 because of its relative resistance to formalin.

Simian Virus 40 could also infect humans, without causing symptoms of disease, following administration by the respiratory route. This became evident when the respiratory syncytial virus vaccine, which also contained as a contaminant SV 40, was given by intranasal route, by nebulization, and also by instillation of the virus into the nostrils (Morris *et al.*, 1961). Out of 35 inoculated volunteers, 3 showed the presence of the Simian Virus 40 in throat swabs taken 7 to 11 days after inoculation; specific neutralizing antibodies appeared in the blood serum of 22 individuals.

Infection with Simian Virus 40 by oral route also occurred in certain instances. Most of the Sabin live poliomyelitis vaccines employed contained only trace amounts of infectious SV 40; a few, however, contained relatively larger amounts, and some among them even close to 10^5 TCD_{50} (Melnick and Stinebaugh, 1962). In several studies no evidence of infection was found in human subjects following ingestion of the oral, live, poliomyelitis vaccine which contained traces of SV 40 as a contaminant (Sweet and Hilleman, 1960. Magrath *et al.*, 1961. Goffe *et al.*, 1961). However, in the study reported by Melnick and Stinebaugh (1962), some of the children who ingested the Simian

Virus 40 present in oral poliomyelitis vaccine later on excreted in their stools very small amounts of live SV 40 for as long as 4 to 5 weeks. This was true when 3- to 6-month-old children and when newborn infants received the Simian Virus 40 with the oral vaccine. Similar observations were reported more recently by Horváth and Fornosi (1964).

> The following personal communication was received by the author (1967) from Dr. Albert B. Sabin:
>
> "With regard to excretion of SV 40 virus by children who ingested oral polio vaccine, Melnick and Stinebaugh (1962) reported only the occasional isolation of small traces of virus in a few children after relatively large amounts were ingested. In our own laboratory, tests were carried out on a total of 149 stool specimens from 45 persons who had ingested about 10^3 to $10^{4.5}$ TCD_{50} of SV 40 virus. No virus was isolated in any instance but it must be said that since the cultures were observed for only a period of two weeks the excretion of small traces of virus could have been missed (Pelon, W., and Sabin, A. B., unpublished data)."

Low-grade Inapparent Infection, but No Symptoms of Disease. These studies established therefore the fact that a low-grade infection with SV 40 may occur in humans following introduction of this virus, particularly by parenteral route, but occasionally also by ingestion. No symptoms of disease have been observed in hundreds of thousands of persons who either had been injected with, or were fed, this agent (Fraumeni *et al.*, 1963). However, the period of observation of humans that received this virus and were observed in the this study was relatively short.

Influence of Dose of SV 40. Oncogenic action of SV 40 depends to a considerable degree on the dose of the virus administered and the route of inoculation. This was demonstrated conclusively in the early experiments carried out on hamsters by Eddy (1962b), Girardi (1963), and more recently also by Diamandopoulos (1975) and their coworkers. Thus, small doses of SV 40, particularly those administered by the oral route, may not induce disease, such as tumors or leukemia, in humans.

Influence of Age on Susceptibility to SV 40, and the Necessity of Long Term Observation

Because of the considerable influence of age on the susceptibility of the host to the oncogenic action of SV 40, it would appear that any oncogenic effect that could be anticipated in humans exposed to this virus could be expected, if at all, only following the inoculation of

the virus into young children and particularly into newborn infants. It is true that in a few instances newborn infants did receive small quantities of live SV 40, inadvertently present in either Salk or Sabin polio vaccines. This occurred prior to the isolation of the SV 40 virus and the recognition of its oncogenic potential; in the early years the presence of the SV 40 contaminant in such vaccines had not yet been recognized. Human exposure to SV 40 occurred in the United States on a large scale between the years 1954 and 1961, perhaps a year or two longer, since vaccines tested in the United States after June 30, 1961, were required to be free of SV 40. Thus far, however, no untoward effects in any of these or other individuals who received SV 40 have been observed. However, a longer observation time, probably two or three additional decades, may be required in order to assess the possible pathogenic and, in particular, oncogenic effect of inoculation of small doses of SV 40 into humans.

> Several groups of children, about 800 of them less than 3 days old and 200 less than 12 days old, received oral polio vaccines which were later found to contain significant amounts of Simian Virus 40. Eight years after this exposure to SV 40, no cancer deaths have been observed among the vaccinated children (Fraumeni *et al.*, 1970). However, this length of time may not be sufficient, and continued surveillance is needed before concluding that SV 40 did not induce tumors following oral inoculation into infants.

Long Term Follow-up of Persons Inadvertently Inoculated with SV 40 as Newborn Infants

In order to determine the possible long term effect of SV 40 on humans, a follow-up study of persons inadvertently inoculated with the Simian Virus 40 as newborn infants was carried out by a group of investigators, with the support of a National Cancer Institute contract (Mortimer *et al.*, 1981).

The following personal communication referring to this study was received by the author in June, 1981, from Dr. Edward A. Mortimer, Case Western Reserve University, Cleveland.

> "There were 1,073 newborn infants who received monovalent oral polio virus vaccine or inactivated polio virus vaccine, inadvertently containing SV 40, during the years 1960–62 (Fraumeni *et al.*, 1970). The follow-up was continued until 1979, at which time the study was discontinued. In 1977, the status of 1,019 (95 percent of the original group) was determined; in 1979 we were able to determine the status of 937 (87 percent). The reason we did less well in 1979 was, of course, the fact that many of the children had dropped out of school or had graduated, making them much more difficult to find.
>
> "Of the original group of 1,073, none was ascertained to have suc-

cumbed to a malignant tumor. One child developed a mixed tumor of the salivary gland 'of low malignancy' at 15 years of age; five years later (January, 1981) there was no evidence of recurrence. By the end of the follow-up period, 15 study subjects were known to be dead, none from a malignant tumor. For each child who died, a cause of death was recognized, usually trauma or congenital heart disease.

"The expected number of deaths in this group was determined for each of the subgroups using age-specific cancer mortality rates for the years 1960 through 1979. The expected number of deaths from cancer came out to be exactly 1.0. Since none occurred, I think the results are quite reassuring. Of course, one does not know what might happen another ten or twenty years later."

Isolation of Simian Virus 40 from Human Tumors

Simian Virus 40 (SV 40) was isolated from malignant metastatic melanomas of a 73-year-old patient and SV 40 T (tumor) antigen was detected in tumor cells of the same patient (Soriano *et al.*, 1974). Simian Virus 40-related (T) antigens as well as virus particles similar in size and structure to the papova-virus group (to which SV 40 belongs) were also found in 3 and possibly in 4 human meningiomas, relatively common, slow-growing brain tumors in man (Weiss *et al.*, 1975). In another study, a virus closely related to SV 40 was isolated from the brains of two patients with progressive multifocal leukoencephalopathy (PML), a rare human demyelinating brain disease (Weiner *et al.*, 1972); in these two cases, the isolated virus was apparently related to, but distinct from, the J. C. virus (Padgett *et al.*, 1971) known to be the etiological agent of that disease (see Chapter 24).

The cases here reviewed represent very few, apparently isolated instances with no documented evidence suggesting that SV 40 was indeed the etiological agent of the reported disease. However, only exceptionally did clinical examination of patients treated for either neoplastic or chronic neurologic conditions include virus studies and immunological surveys aiming at the detection of a possible etiological relationship of their disease to Simian Virus 40 (Shah, 1972. Shah and Nathanson, 1976).

Elimination of SV 40 from Vaccines

Following the isolation of the Simian Virus 40, and the recognition of its oncogenic potential, it became of utmost importance to test vaccines produced for human use for presence of the vacuolating agent (SV 40), and to free such vaccines from any traces of live SV 40. A provision of U.S. federal regulations governing the prepa-

ration of poliomyelitis virus and adenovirus vaccines excludes fro
sale all vaccines containing an adventitious agent of unknown pat
ogenicity. Upon discovery of SV 40 in killed poliovirus and adenovir
vaccines, the elimination of this contaminating virus became impe
ative, and several methods have been described for either selectivel
destroying SV 40 or for employing for the preparation of vaccin
cell cultures free from the vacuolating agent. SV 40 is relatively r
sistant to amounts of formaldehyde sufficient to inactivate eith
poliovirus or adenoviruses (Sweet and Hilleman, 1960b. Gerber *et al*
1961. Eddy, 1964. Girardi, 1965). However, SV 40 is susceptible t
inactivation by visible light in the presence of toluidine blue (Hia
et al., 1962), or by heating at 50°C for one hour in the presence
molar solution of magnesium or calcium salts (Wallis and Melnic
1961). It was also observed (Hayashi and LoGrippo, 1962) that Simia
Virus 40 is more susceptible to inactivation by beta-propiolacton
than either the poliomyelitis virus or the adenoviruses, and that th
chemical is effective in destroying SV 40 in poliomyelitis virus an
adenovirus vaccines without impairing their potency.

Later it became mandatory that virus pools prepared for produ
tion of vaccines be tested and found free from SV 40 contaminatio
prior to inactivation of such vaccines with formaldehyde. The metho
of choice, however, to prevent contamination of vaccines with SV 4
is to propagate the poliomyelitis virus, or another virus which is t
be used for the preparation of vaccines, on cell cultures free from th
vacuolating agent. Many rhesus monkey kidney cell cultures contai
the Simian Virus 40, but some are relatively, or even completely, fr
from such contamination. Traces of SV 40, if present in such monke
kidney cell cultures, can be neutralized by specific anti-SV 40 immun
serum. Careful tests are required to determine whether the cell cu
tures which are to be used for propagation of virus are free fro
contamination with the Simian Virus 40. Such tests include inc
bation of fluid from the cultures on green monkey (*Cercopithecu*
kidney cells for prolonged periods of time, in order to determi
whether the characteristic vacuolating effects of SV 40 may not b
come apparent. Another measure consists of successive serologic
tests performed on monkeys serving as prospective donors for th
preparation of monkey kidney cell cultures, in order to determine th
possible presence of specific antibodies directed against SV 40. An
mals with antibodies are likely to provide contaminated tissues. O
the other hand, monkeys which remain free from antibodies for
number of weeks, while maintained under isolated conditions, shoul
provide tissues which will not yield SV 40 in cell cultures.

Finally, it may also be possible to employ monkey cell cultur originating from species known to be free from the Simian Virus 40 for the propagation of poliomyelitis or other viruses.

Lépine and Sautter at the Pasteur Institute in Paris reported (1962) that for the preparation of polio vaccines they have propagated the poliomyelitis virus on kidney cells obtained from African monkeys (*Cynocephalus Papio papio*) which are not natural carriers of the Simian Virus 40. Furthermore, the vaccines prepared at the Pasteur Institute were carefully tested for absence of SV 40. In view of the fact that the vacuolating agent is relatively resistant to formol, the vaccines were inactivated by beta-propiolactone.

Oncogenic Potential of Simian Virus 40. Its Significance and Theoretical Implications

The significance of the oncogenic potential of the vacuolating virus (SV 40) is considerable. The observation that a latent virus indigenous in monkey kidney cells, and apparently harmless for its carrier host species, can induce a high incidence of malignant, progressively growing tumors when inoculated into newborn animals of another species is striking. Similar observations have been recorded in studies dealing with adenoviruses, a group of agents either remaining latent or producing only a mild and transient disease in their human carriers, but at the same time capable of inducing tumors in another species, i.e., in hamsters. This pattern of biological behavior of a potentially pathogenic agent does not seem to be an isolated phenomenon in nature. Many parasitic, bacterial, and viral agents may either remain latent and propagate without causing symptoms of disease, or cause only a transient, mild illness in one or more species of carrier hosts, whereas they may be capable of causing symptoms of serious, frequently fatal, disease in certain other animal species. Rocky Mountain spotted fever, certain forms of epidemic encephalitis, herpes, yellow fever, distemper, and many other transmissible diseases of animals and man can be quoted as examples. In this respect, therefore, oncogenic viruses do not fundamentally differ from other pathogenic agents.

The obvious question arises whether there exist viruses, other than Simian Virus 40 in monkeys, or adenoviruses in humans, which may belong to this group, i.e., which would be latent under natural life conditions, or cause only a transient disease in their natural carrier hosts, but could be potentially oncogenic for some other species? Are there perhaps some other as yet undetected, latent, but potentially oncogenic viruses in tissue culture cells, or in other live media, such as embryonated chicken eggs, now routinely employed for preparation of vaccines for animals or for humans?

Herpesviruses also belong to this important group of potentiall oncogenic agents. In most instances they remain latent, causing n harm to their carrier hosts; in some instances they may cause tran sient, inflammatory lesions; under certain conditions, however, the may also cause malignant lymphomas and leukemia.

These observations are described in Chapter 28 on Leukemia and Lymphomas in Nonhuman Primates. The interested reader is also re ferred to Chapter 26 on Burkitt Lymphoma.

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CHAPTER 24

Isolation of a Polyoma-Like Virus (J. C.) from a Rare Human Brain Disease

Progressive Multifocal Leukoencephalopathy (PML)

Progressive Multifocal Leukoencephalopathy. Observation of a New Disease Syndrome. In the course of post-mortem examination of brains of patients at the Massachusetts General Hospital in Boston, Åström, Mancall, and Richardson (1958) observed cases of an unusual demyelinating disease of the cerebral white matter; the lesions were characterized by widely disseminated, generally oval, small perivascular foci of destruction of myelin sheaths with relative sparing of axis cylinders. Three such cases were reported and recognized as a syndrome, and designated "progressive multifocal leukoencephalopathy." Two of these cases were observed in patients with chronic lymphatic leukemia and the third case in a patient with Hodgkin's disease. Shortly thereafter additional cases of a similar disease syndrome were reported by Cavanagh and his coworkers (1959), Lloyd and Urich (1959), Sibley and Weisberger (1961), and Richardson (1961).

Neurological symptoms in afflicted persons were progressive over an average period of three to four months and included hemiparesis, visual disturbances, aphasia, mental changes, and deterioration of intellect.

> The early lesions of progressive multifocal leukoencephalopathy are multiple small areas of demyelination, usually at the border between the white matter and cortex of the cerebrum. The pathognomonic cells, altered oligodendrocytes, are most prominent in and around the early lesions. These oligodendrocytes have enlarged nuclei that stain abnormally and may contain inclusion bodies. The early lesions enlarge, coalesce and may become necrotic. In late lesions few oligodendrocytes are seen, but reactive astrocytes, macrophages, and giant astro

The J. C. virus was subsequently isolated from more than 30 additional cases of progressive multifocal leukoencephalopathy in humans (Zu Rhein, 1969. Narayan *et al.*, 1973. Walker *et al.*, 1973. Padgett *et al.*, 1976); it is now assumed that this virus is the actual cause of this rare human brain disease. Whether the progressive multifocal leukoencephalopathy in humans can be caused only by the J. C. virus is a question which up to the present time remains open, because in at least 3 cases other variants of a polyoma-like virus having a rather close relation to the Simian Virus 40 have been isolated (Weiner *et al.*, 1972. Weiner and Narayan, 1974. Sack *et al.*, 1973. Narayan *et al.*, 1973).

Properties of the J. C. Virus

Based upon characteristics such as size, stability, intranuclear multiplication, etc., the J. C. virus fits into the polyoma and SV 40 subgroup of the family of papovaviruses. The J. C. virus causes agglutination of human, guinea pig, and chicken erythrocytes at 4°C. It seems not to agglutinate sheep, African green monkey, rhesus monkey, or hamster erythrocytes. Although the host range has not been thoroughly explored, the virus shows no evidence of causing acute disease in rabbits, guinea pigs, hamsters, mink, or rhesus monkeys. It was most important to distinguish it from human papilloma virus and from SV 40, which is a virus that infects the rhesus monkey. The J. C. virus is antigenically unrelated to the papilloma virus and has a hemagglutinin that the papilloma virus lacks. The hemagglutinin is also important in distinguishing it from the SV 40 virus; but, in addition, the J. C. virus does not multiply in African green monkey kidney cells or in other cells that are commonly used for cultivation of the SV 40 virus. However, it does appear that SV 40 and J. C. viruses share some minor antigens and that the J. C. virus is therefore related antigenically to the SV 40 virus (Walker *et al.*, 1973, 1974. Padgett *et al.*, 1976).

Serological studies have demonstrated that the J. C. virus is widely present in a large segment of the normal human adult population. Sera from 406 people of various ages collected in the state of Wisconsin were assayed for antibodies against the J. C. virus; the hemagglutinination-inhibition test was used for the testing. About 69 percent of the adults tested had significant levels of antibodies against the J. C. virus in their sera. Infection with the J. C. virus appears to be a common event, and most infections occur during childhood. As yet, nothing is known about the character or the usual course of the primary infection (Padgett and Walker, 1973).

Serological Identification of PML by Electron Microscopy

Culture methods are time consuming, and viral identification in general is more easily achieved by immunofluorescent staining of frozen sections or by the technique of immune electron microscopy (Albert and Zu Rhein, 1974). In the latter, viruses which have been physically extracted from fresh preparations are being incubated with various known specific antisera, and the appearance of antibody molecules, visible in the electron microscope, identifies the virus. Penney and his coworkers at the Department of Neurology, Johns Hopkins University, Baltimore, first applied this technique successfully (1972) for identification of a virus extracted directly from a case of progressive multifocal leukoencephalopathy.

Oncogenic Potency of the J. C. Virus for Newborn Hamsters

No definite evidence has yet been presented to suggest that the J. C. virus has an oncogenic potency for human hosts. However, the virus was found to be oncogenic when inoculated into newborn hamsters or into certain species of nonhuman primates; other animal species may also be susceptible. The fact that the J. C. virus is oncogenic was demonstrated in experiments in which newborn hamsters were inoculated with the J. C. virus. Within 6 months after inoculation, 53 out of 63 hamsters (83 percent) developed brain tumors (gliomas). In most instances multiple tumors developed, often of different histologic appearance, such as medulloblastomas, glioblastomas, or unclassified primitive tumors. None of 39 hamsters inoculated with control tissue culture fluid developed tumors (Walker *et al.*, 1973). In another experiment in which newborn hamsters were inoculated with the J. C. virus by intraperitoneal and subcutaneous routes, 42 out of 75 animals developed tumors within 14 months. Many organs were involved. Most animals had multiple tumors, and 10 hamsters developed subcutaneous tumors; all tumors were sarcomas (Padgett and Walker, 1976).

Varakis and Zu Rhein (1976) reported a brain tumor, pineocytoma, which developed in a Syrian hamster, 5 months after intracerebral inoculation of the J. C. virus. Development of peripheral neuroblastomas following injection of newborn hamsters with the J. C. virus was also reported (Varakis *et al.*, 1978).

Following intraocular inoculation of the J. C. virus into newborn hamsters, 20 percent of the inoculated animals developed intraocular tumors; most of these tumors were identified as retinoblastomas (Ohashi *et al.*, 1978).

Induction of Brain Tumors in Owl Monkeys with the J. C. Virus. Adult owl monkeys (*Aotus trivirgatus*) were inoculated intracerebrally, subcutaneously, and intravenously with the J. C. virus. Two of the four inoculated monkeys developed brain tumors 16 and 25 months after inoculation, respectively. An astrocytoma (resembling a human glioblastoma multiforme) was found in the left cerebral hemisphere and brainstem of one monkey; the second monkey developed a malignant tumor in the left cerebral hemisphere containing both glial and neuronal cell types (London *et al.*, 1978).

* * *

The J. C. virus represents another example of an oncogenic virus capable of inducing a non-neoplastic disease in man. Similar observations made on adenoviruses are described in the next chapter of this monograph. Certain types of human adenoviruses can be latent or may induce only a transient infectious disease in humans, such as conjunctivitis, enteritis, or pharyngitis; yet, the same viruses can induce progressively growing sarcomas following inoculation into newborn hamsters, rats, or *Mastomys*.

THE B. K. VIRUS (SIMILAR TO THE J. C. VIRUS) ISOLATED FROM URINE OF PATIENTS UNDERGOING IMMUNOSUPPRESSIVE THERAPY

In 1971 Sylvia D. Gardner and her coworkers, at the St. Mary's Hospital in London, England, isolated a virus resembling the polyoma virus from the urine of a 39-year-old Sudanese male patient who received a kidney transplant from his brother and who was under immunosuppressive (prednisone and azathioprine) therapy. The patient developed an obstruction at the site of the ureter implantation into his bladder, which had to be relieved by surgical procedure. From several urine samples collected from this patient, a virus was isolated and propagated in *vero* cells, a cell-line derived from African green monkey kidney cultures. The virus, designated by the letters B. K., representing the initials of the patient from whom it was isolated, had the size and morphology of a papovavirus of the polyoma subgroup with a minor antigenic relationship to the SV 40 virus. Subsequent isolations of virus strains indistinguishable from the B. K. virus have been made from urine of other immunosuppressed renal allograft recipients in England (Coleman *et al.*, 1973), South Africa (Lecatsas *et al.*, 1973), and Switzerland (Jung *et al.*, 1975), as well as from urine of children and adults receiving chemotherapy for leukemia, lymphomas, or other neoplastic diseases (Reese

et al., 1975. Zu Rhein and Varakis, 1974a). In one case, the B. K. virus was isolated from a brain tumor, a reticulum cell sarcoma of a child (Takemoto *et al.*, 1974).

It is now quite apparent that the B. K. virus can be isolated without difficulty from patients who receive immunosuppressive treatment, such as those who have had renal transplants, or from patients who have malignant disease and are treated by chemotherapeutic agents. However, up to the present time the B. K. virus has not been isolated from urine of normal adults or children.

On the other hand, serological surveys determined the presence in blood plasma of antibodies directed against the B. K. virus in large segments of the human population in this country and abroad. In most instances, the hemagglutination-inhibition test has been used to determine the presence of antibodies. A study of over 500 sera from all age groups in England established that infection occurred very early in life. The rate of serum conversion was such that 73 percent of the 4-to 6-year-old age group had antibodies to this virus (Gardner, 1973). Similar results were obtained in a survey carried out in the United States (Shah *et al.*, 1973). However, the B. K. virus did not seem to be associated with symptoms of disease in any of the patients in which it was found.

Some Properties of the B. K. Virus. The B. K. virus produces cytopathic effects in primary rhesus monkey kidney cells (Gardner *et al.*, 1971) and in primary African green monkey kidney cells (Takemoto and Mullarkey, 1973). It agglutinates erythrocytes from humans, guinea pigs, and young chickens but not those from African green monkeys or from several strains of mice tested (Gardner *et al.*, 1971. Mäntyjärvi *et al.*, 1972).

Negatively stained extracellular B. K. virus particles have a diameter of between 40.5 and 44 nm. They have an icosahedral structure with 72 capsomers. Morphologically, they are indistinguishable from the J. C. virus and from the polyoma virus particles (Gardner *et al.*, 1971).

Oncogenic Potency of the B. K. Virus

Transformation of Cells in Tissue Culture. The B. K. virus was reported to transform hamster cells in tissue culture (Major and Di Mayorca, 1973).

Induction of Tumors in Hamsters with the B. K. Virus. When 52 newborn hamsters were inoculated subcutaneously with the B. K. virus, only one developed an undifferentiated sarcoma at the site of inoculation, after 8 months (Shah *et al.*, 1975). It thus appeared that the B. K. virus has a low oncogenic potential. However, subsequent studies demonstrated that under proper experimental conditions, the B. K. virus could induce a high incidence of tumors in laboratory animals. When the B. K. virus was inoculated intravenously into three-week-old Syrian golden hamsters, 8 percent of the animals developed tumors after a latency varying from 3 to 9 months; among the tumors observed were ependymomas, carcinomas of the pancreatic islets, osteosarcomas, adenocarcinomas, angiosarcomas, and lymphomas (Corallini *et al.*, 1978).

In another study, newborn hamsters were inoculated intracerebrally with purified and concentrated B. K. virus preparations from a single stock of Gardner's original strain. Most of the inoculated hamsters developed a variety of tumors after a latency of 3 to 9 months. The most frequent types of induced tumors were ventricular brain tumors (choroid plexus papillomas and ependymomas), malignant insulinomas, and osteosarcomas (Uchida *et al.*, 1976, 1979).

The R. F. Virus

Dougherty and Di Stefano reported in 1974 the isolation from urine of a renal transplant patient of another virus resembling the polyoma virus, and very similar in its morphology and properties to the J. C. and B. K. viruses. They designated this virus by the letters R. F., representing the initials of the name of the patient from whom it was isolated. The R. F. virus proved to be highly oncogenic for hamsters; when inoculated into newborn hamsters it induced subcutaneous sarcomas in 50 percent of the inoculated animals after a latency varying from 18 to 48 weeks (Dougherty, 1976). Antibodies to the R. F. virus were observed in 94 percent of 400 adult human sera tested; however, no tumors in humans related to the presence of this virus have been thus far identified. Up to the present time, no disease in humans has been observed to be associated with the presence of the R. F. virus.

It is very possible that the B. K. and R. F. viruses represent closely related, if not identical, virus strains. They share most of their biological properties.

* * *

The recovery of polyoma-like viruses, such as the B. K. virus or the R. F. virus, from human urine is of considerable interest.

The polyoma virus, which belongs to the same group, can readily be recovered from urine of mice which carry that virus (Rowe *et al.*, 1960). It is also of interest that in most instances, under natural life conditions, mice carrying the polyoma virus do not develop tumors.

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History of Sabin attenuated poliovirus oral live vaccine strains*

A. B. Sabin† *and L. R. Boulger*‡

The full data concerning the history of attenuated poliovirus strains developed by one of us (Sabin, 1965) for vaccine production do not appear in a single journal. Over the past few years we have had frequent requests for the details such as isolation and attenuation and accordingly we felt that bringing the data together in the report below would be both helpful and informative to those involved in the production and control of poliovirus vaccine (oral) prepared from these strains.

Type 1 LS-c, 2ab/KP$_2$ (Sabin Original Virus = SO)

The Mahoney virus was isolated in 1941 by Drs Fancis and Mack from the pooled faeces of three healthy children in Cleveland.

Drs Li and Schaeffer received the strain form Dr Salk after it had undergone 14 monkey *in-vivo* and two monkey *in-vitro* testicular tissue culture passages. Li & Schaeffer (1954) subjected this strain, Monk14 T2 (Mahoney strain), to a further nine similar *in-vitro* passages. From Monk14 T11 they established four separate virus lines by further passages in monkey testicular tissue and kidney cell cultures, by passages in the central nervous systems of white mice usually by the intraspinal route of injection and by alternate passages in the skin of rhesus or cynomolgus monkeys and tissue cultures. For the intradermal injections, ten 0·1 ml amounts of undiluted culture fluid were introduced adjacent to each other into the shaved skin of the abdomen. The four passaged strains were designated LS, LS-a, LS-b and LS-c and as they continued to grow in cell culture

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they were differentiated by their host reactions. LS and LS-a reacted similarly in that they were mouse and monkey spinal cord variants. LS-b acted as a mouse cerebral strain, but LS-c was a 'non-neurotropic' strain for mice and monkeys by either route. It was derived from the thirty-third consecutive *in-vitro* passage of Monk14 T2 (the first 15 in testicular tissue and the subsequent 18 in kidney cells), then by alternate passage in monkey skin (the first two of these were *in-vivo* rhesus passages and the later ones were performed in cynomolgus monkeys) and monkey kidney cell cultures. The LS-c strain is Monk14 MS10 T43 level.

The LS-c strain underwent five passages in cynomolgus monkey kidney cell cultures, including three terminal dilution passages, prior to submission to a series of three consecutive single plaque passages (Sabin, 1956). The progeny of ten selected individual plaques were tested for neurovirulence in cynomolgus monkeys inoculated intraspinally with 10^6, 10^5 and 10^4 tissue culture infective virus doses, and the LS-c, 2ab strain was selected because it possessed the optimum properties. The original type 1 virus (SO) was prepared by two further passages in cynomolgus monkey kidney cell cultures and designated LS-c, 2ab KP_2 of 10/10/56. Its volume was 100 ml, the pH was 8·2, the titre was 7·9 $\log_{10}$ $TCID_{50}$ per ml (Tissue Culture Infectious Dose 50%) and only one cynomolgus monkey out of five receiving undiluted material intraspinally exhibited slight paralysis. The two groups of animals inoculated with suspension diluted 1/10 and 1/100 showed no paralysis. At the end of 1956 Merck, Sharp & Dohme Research Laboratories prepared a Lot of 25 l by one passage of the original virus in rhesus monkey kidney cell cultures. This material was designated LS-c, 2ab KP_3 (MSD, SOM or SO+1), and aliquots were used for the world-wide field trials before it was licensed as the Sabin original vaccine, and as the Sabin seed virus for the production of vaccine.

Type 2 P712, Ch, 2ab KP_2 (Sabin Original Virus = SO)

The original P712 virus was a naturally occurring strain of poliovirus possessing low neurovirulence for cynomolgus monkeys by the intraspinal route (Sabin, 1956). The faeces from a number of healthy children in Louisiana were sent by Drs Fox and Gelfand to Dr Sabin, who isolated the P712 strain from one of these specimens.

Because of its low initial neurovirulence for monkeys it was passaged four times in cynomolgus monkey kidney cell cultures, three of which were terminal dilution ones. The progeny from a number of plaques were obtained, and nine were submitted to three consecutive plaque passages (Sabin, 1957). The purified plaque progeny with the least neurovirulence for cynomolgus monkeys, inoculated intraspinally as with type 1 progeny, was fed to chimpanzees and the excreted strain possessing the least residual neurovirulence (P712, Ch) was further purified by three consecutive passages from single plaques, and the strain designated P712, Ch, 2ab selected as the vaccine virus. The original type 2 virus (SO) was prepared by two further passages in cynomolgus monkey kidney cell cultures and named P712, Ch, 2ab KP_2 of 10/10/56. Its volume was 100 ml, the pH was 8·2, the titre was 7·3 $\log_{10}$ $TCID_{50}$ per ml and none of the three groups of five cynomolgus monkeys each inoculated with 0·1 ml amounts of undiluted virus suspension and suspension diluted tenfold and hundredfold showed any degree of paralysis. As with the type 1 attenuated poliovirus, Merck, Sharp and Dohme Research Laboratories made a 25 l Lot by one passage of the original type 2 virus in rhesus cultures. This is the P712, Ch, 2ab KP3 (MSD, SOM or SO+1) and aliquots were used for the field trials before it was licensed as the Sabin original vaccine and as the Sabin seed virus.

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Williams v.

Type 3 Leon $12a_1b$ KP3 (Sabin Original Virus = SO)

The Leon virus was obtained from the brain-stem and spinal cord of an 11-year-old boy, who had died of bulbo-spinal poliomyelitis in Los Angeles in 1937. It was isolated by Drs Kessel and Stimpert in rhesus monkeys and maintained in the same species by the intracerebral route for 20 subsequent passages in 1951. It underwent eight further passages in rhesus monkey testicular tissue culture before the strain was sent by Dr Melnick to Dr Sabin (Sabin *et al.*, 1954). After three passages in cynomolgus monkey kidney cell cultures the virus produced prostrating paralysis within 4–6 days in each of four intracerebrally inoculated cynomolgus monkeys. Thirty rapid passages at *c.* 24-h intervals, using large inocula (10^5 to 10^6 $TCID_{50}$) were carried out in cynomolgus kidney cultures. These were succeeded by three terminal dilution passages, followed by one passage using a large inoculum of the progeny of the third terminal dilution. This strain, Leon KP34, exhibited a marked reduction in its neurovirulence in that none of the 28 cynomolgus monkeys inoculated intracerebrally with 7·2 $\log_{10}$ $TCID_{50}$ per ml developed either clinical or histological poliomyelitis. The progeny from nine selected plaques, after purification by three consecutive plaque passages, was subjected to the neurovirulence test in three groups of cynomolgus monkeys inoculated intraspinally with 6·0, 5·0 and 4·0 $\log_{10}$ $TCID_{50}$ of virus. The progeny designated as $12a_1b$ showed the least neurovirulence and was selected for the production of vaccine (Sabin, 1956). This strain was passaged three times in cynomolgus monkey kidney cell cultures to give the original type 3 virus (SO) named Leon $12a_1b$ KP_3 of 10 10 56 (Sabin 1957). The volume was 10 ml, the pH was 6·8, the titre was 6·5 $\log_{10}$ $TCID_{50}$ per ml and three groups of five cynomolgus monkeys were each inoculated intraspinally with 0·1 ml amounts of undiluted virus suspension as well as 10^{-1} and 10^{-2} dilutions. The monkeys receiving the undiluted material and those inoculated with suspension diluted one hundredfold remained symptomless, whereas one of the five animals which had the tenfold dilution showed minimal clinical signs and focal histological poliomyelitis adjacent to the site of injection in the lumbar cord. Merck, Sharp and Dohme prepared a Lot of 25 l by one passage (as with types 1 and 2) using the original type 3 virus (SO). This Lot is Leon $12a_1b$ KP_4 (MSD, SOM or SO+1) and was used in the field trials before being licensed as the Sabin original vaccine and as the Sabin seed virus.

The three types of the large Lots produced by Merck, Sharp and Dohme in rhesus monkey kidney cell cultures contained SV40 (W.H.O., 1969).

Alternative Leon $12a_1b$ vaccine strains

The original SO type 3 virus which was free of SV40 was supplied to Lederle Laboratories, who prepared their seed Lot (No. 45B-85) by one passage of the original virus, previously mixed with SV40 antiserum, in cercopithecus monkey kidney cell cultures; this was then used to make a larger Lot, designated No. 3-393. This material was approved by the Division of Biologics Standards, United States Public Health, and it represents passage level SO+2 (W.H.O., 1969). Aliquots have been used by some manufacturers for the production of working seed, i.e. SO+3 so that their vaccines represented SO+4

The original SV40-free type 3 virus (SO) was also given to other manufacturers, of which two prepared working seed in one passage, i.e. SO+1, so that their vaccines represented the second cell culture passage level or SO+2. The other producers made a further cell culture passage before preparing the working seed, i.e. SO+2 meaning their vaccines were third passage level or SO+3. Another manufacturer prepared their working seed

Produc [illegible]
Lede [illegible]
Williams v. Lederle

by two consecutive cell culture passages of the SOM virus, i.e. SO+3 so that their vaccines were fourth passage level or SO+4.

The majority of the manufacturers receiving the Sabin seed virus (SOM or SO+1) types 1, 2 and 3 made their working seeds by one passage to free SOM from SV40. Hence their vaccines were third passage level or SO+3 (W.H.O., 1969). One producer (Chumakov, Dzagurov, Lashkevich, Grachov, Mironova, Ralph & Elbert, 1964) freed SOM+2 from SV40 by 2 heat treatments at 34°C in the presence of 1 M $MgCl_2$, after which six plaques were selected and grown on vervet monkey cell cultures and pooled to form working seed. This was then subjected to one more heat treatment at 34°C in the presence of magnesium chloride. The seed virus represents SOM+5 or SO+6 so that the vaccines were SOM+6, i.e. SO+7. Finally, another manufacturer (Stones, MacDonald, McDougall & Ramsbottom, 1964) grew SOM type 3 in vervet monkey cells in the presence of SV40 antiserum. This material was then subjected to phenol extraction and plaque purification to form an RNA working seed equivalent to SOM+4 and RNA vaccines to SOM+5, i.e. SO+6.

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Produced by
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Simian Virus Lab. 12.18.61

Pathology Lab.

Dr. E. Jungherr
Dr. R. Percival

Dr. Bittle ✓
Dr. Cabasso
Mr. Coggeshall
File

Virus Isolations

First and second passages in RMK, RbK and CMK tissue cultures were performed on the following CNS specimens:

Type: III Batch: 3107 (Seed)

Route	Dilution	Path. #	Leg #	Chest #	Viruses Isolated
I.C.	10^{-0}	W61/2414	C-999	F1-361	Polio-like agent isolated from brain and cord in RMK and CMK. Identity will be checked.
I.C.	10^{-0}	W61/2416	D-2	F1-676	Vacuolating agent "SV_{40}" isolated from brain in 2nd passage CMK.

	Polio Ab. Level		
	Type I (1:8 Final)	Type II	Type III
C-999 (Pre-bled) 8.1.61	-	-	-
(Post-bled) 10.16.61	-	-	>1:256
D-2 (Pre-bled) 8.9.61	-	-	-
(Post-bled) 10.10.61	-	-	-

G. E. Stiles

GES:df

INTEROFFICE CORRESPONDENCE

OCT 31 1961

Pearl River, N.Y. October 30, 1961
OFFICE DATE

TO:

ATT'N. OF: Dr. J. Bittle

COPY TO:
Dr. V. Cabasso
Dr. I.S. Danielson
Mr. H. Ostrom
Dr. H.D. Piersma
Mr. G. Stiles

SUBJECT: Request for Virological Examination

REFERENCE:

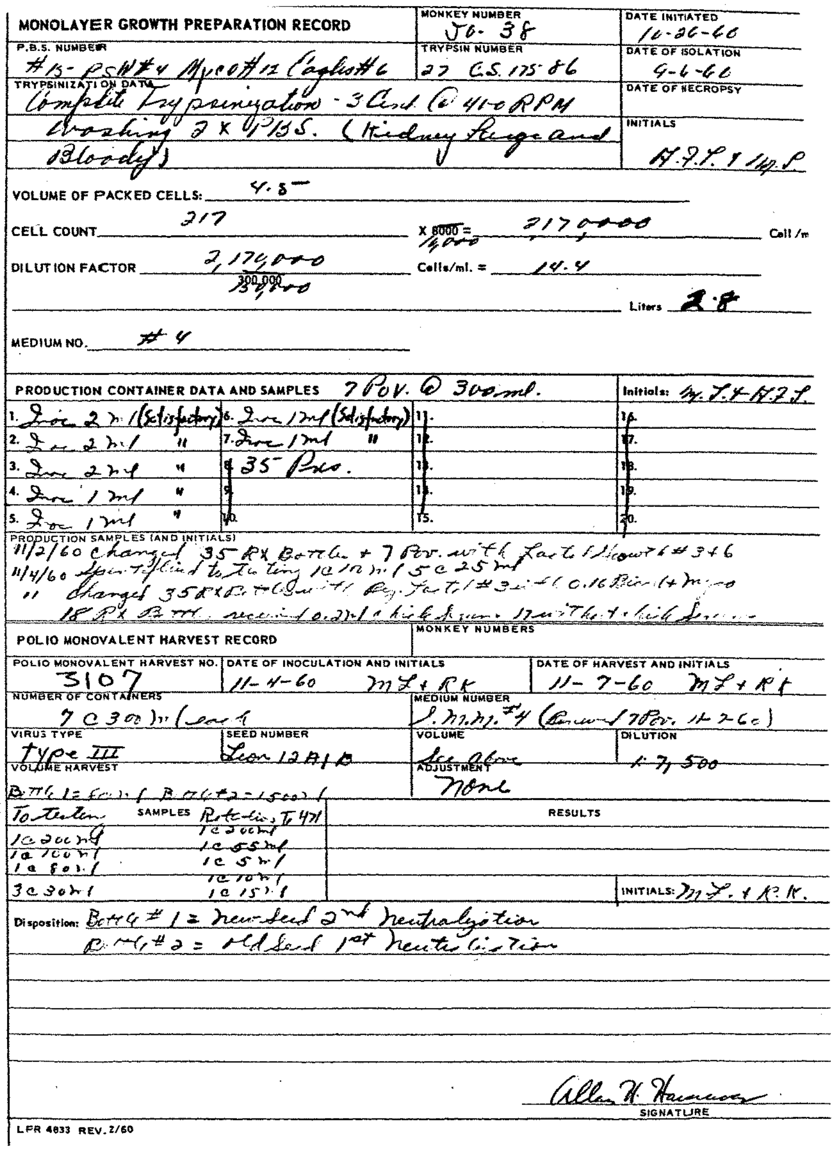
We should like to request that virus isolation be attempted on the following frozen tissues:

Type	Batch	Route	Dilution	Path. #	Leg #	Chest #	Remarks
III	3107 (seed)	I.C.	10^{-0}	W61/2414	C-999	F1-361	Severe lesions throughout CNS.
III	3107 (seed)	I.C.	10^{-0}	W61/2416	D-2	F1-676	Comparable animal to W61/2414, with no lesions in CNS.

We should also like to have the polio antibody titered from the post-inoculation bleedings to determine if there is a marked difference in these animals in humoral antibody.

R. C. Percival

RCP:hg



MONOLAYER GROWTH PREPARATION RECORD

MONKEY NUMBER: J6-38

DATE INITIATED: 10-26-60

P.B.S. NUMBER: #13-PSW#4 Myco#12 Caglio#6

TRYPSIN NUMBER: 27 C.S. 175 86

DATE OF ISOLATION: 9-6-60

TRYPSINIZATION DATA: Complete Trypsinization - 3 Cent. @ 410 RPM Washing 2 X P.B.S. (Kidney Large and Bloody)

DATE OF NECROPSY:

INITIALS: H.T.L. & M.P.

VOLUME OF PACKED CELLS: 4.5

CELL COUNT: 317 X ~~6000~~ 14,000 = 2170000 Cell /m

DILUTION FACTOR: 2,174,000 / 300,000 150,000 Cells/ml. = 14.4

Liters 2.8

MEDIUM NO. #4

PRODUCTION CONTAINER DATA AND SAMPLES 7 Pov. @ 300 ml. Initials: M.T.L. H.T.L.

1. Inoc 2 ml (Satisfactory)	6. Inoc 1 ml (Satisfactory)	11.	16.
2. Inoc 2 ml "	7. Inoc 1 ml "	12.	17.
3. Inoc 2 ml "	8. 35 Pro.	13.	18.
4. Inoc 1 ml "	9.	14.	19.
5. Inoc 1 ml "	10.	15.	20.

PRODUCTION SAMPLES (AND INITIALS)

11/2/60 changed 35 RX Bottle + 7 Pov. with [illegible] #3+6
11/4/60 Spent fluid to testing 1c 10 ml 5c 25 ml
" Changed 35 RX Bott. with Reg. [illegible] #3 with 0.16 Bicarb + Myco
18 RX Bott. received 0.2 ml [illegible]

POLIO MONOVALENT HARVEST RECORD

MONKEY NUMBERS:

POLIO MONOVALENT HARVEST NO.: 3107

DATE OF INOCULATION AND INITIALS: 11-4-60 M.L. + R.K.

DATE OF HARVEST AND INITIALS: 11-7-60 M.L. + R.K.

NUMBER OF CONTAINERS: 7 @ 300 ml each

MEDIUM NUMBER: S.M.M. #4 (Renewed 7 Pov. 11-2-60)

VIRUS TYPE: Type III

SEED NUMBER: Leon 12A1B

VOLUME: See Above

DILUTION: 1:7,500

VOLUME HARVEST: Bott 1 = 600 ml Bott #2 = 1500 ml

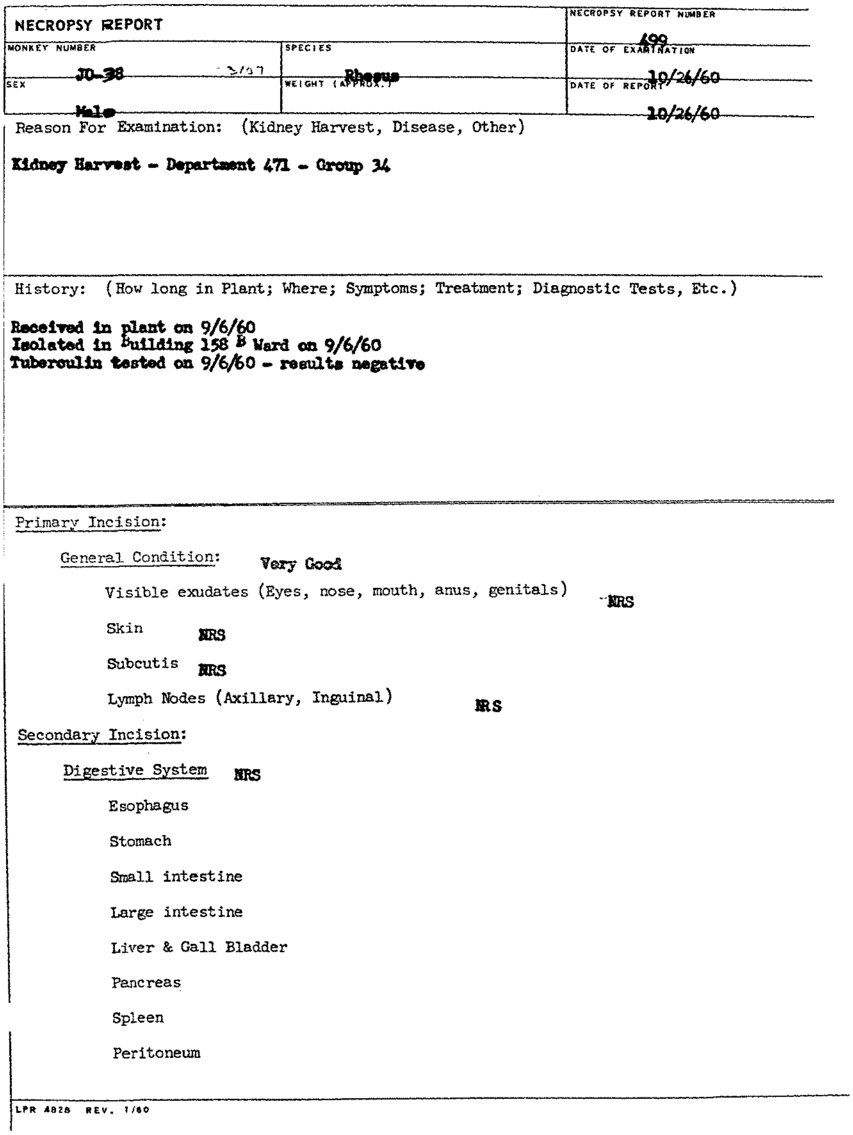
ADJUSTMENT: None

To testing	SAMPLES Retention, T 471	RESULTS
1c 200 ml	1c 200 ml	
1c 100 ml	1c 55 ml	
1c 80 ml	1c 5 ml	
	1c 10 ml	
3c 30 ml	1c 15 ml	INITIALS: M.L. + R.K.

Disposition: Bott #1 = New Seed 2nd Neutralization
Bott #2 = Old Seed 1st Neutralization

Allan W. Hanson
SIGNATURE

LFR 4833 REV. 2/60



NECROPSY REPORT		NECROPSY REPORT NUMBER 499
MONKEY NUMBER JO-38	SPECIES Rhesus	DATE OF EXAMINATION 10/26/60
SEX Male	WEIGHT (APPROX.)	DATE OF REPORT 10/26/60

Reason For Examination: (Kidney Harvest, Disease, Other)

Kidney Harvest - Department 471 - Group 34

History: (How long in Plant; Where; Symptoms; Treatment; Diagnostic Tests, Etc.)

Received in plant on 9/6/60
Isolated in Building 158 B Ward on 9/6/60
Tuberculin tested on 9/6/60 - results negative

Primary Incision:

General Condition: **Very Good**

Visible exudates (Eyes, nose, mouth, anus, genitals) **NRS**

Skin **NRS**

Subcutis **NRS**

Lymph Nodes (Axillary, Inguinal) **NRS**

Secondary Incision:

Digestive System **NRS**

Esophagus

Stomach

Small intestine

Large intestine

Liver & Gall Bladder

Pancreas

Spleen

Peritoneum

LPR 4828 REV. 1/60

OCT 24 1961

Pearl River, N.Y. October 23, 1961

Mr. E. Ostrom

Intramuscular Seed Neurovirulence Tests

Mr. S. Alston
Dr. J. Bittle
Dr. L. S. Danielson
Mrs. M. Priestly

The following seed should be retested by the intramuscular route giving 5.0 ml of the material to each animal. No dilution should be made.

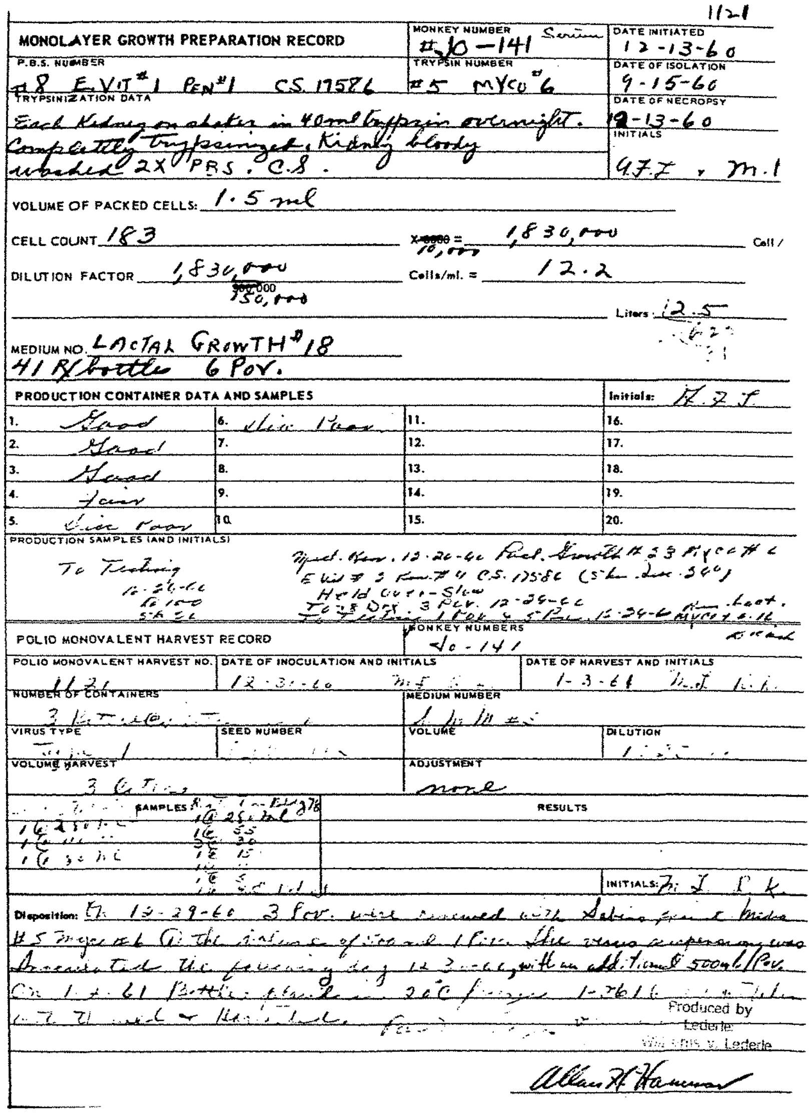
Seed	Original Titer	Titer After 1 to 5 Dilution
1121-1122-1124 (45B-51)	$10^{7.5}$TCID$_{50}$/ml	$10^{6.51}$TCID$_{50}$/ml

This seed must be retested in as much as after the dilution we originally made the titer resulting was too low to result in a total dosage of $10^{7.7}$TCID$_{50}$ per animal.

We should also like to request that seed #2107 be retitrated in as much as the 1 to 5 dilution resulted in a titer of $10^{6.9}$TCID$_{50}$/ml which is 0.1 log lower than required to result in a total per animal dosage of $10^{7.7}$TCID$_{50}$.

R. C. Percival

RCP:bg



1121

MONOLAYER GROWTH PREPARATION RECORD

MONKEY NUMBER: #10-141 Serum

DATE INITIATED: 12-13-60

P.B.S. NUMBER: #8 E. VIT #1 PEN #1 CS. 17586

TRYPSIN NUMBER: #5 MYCO #6

DATE OF ISOLATION: 9-15-60

TRYPSINIZATION DATA: Each Kidney on shaker in 40ml trypsin overnight. Completely trypsinized, Kidney bloody washed 2X PBS. C.S.

DATE OF NECROPSY: 12-13-60

INITIALS: H.F.T. + M.I

VOLUME OF PACKED CELLS: 1.5 ml

CELL COUNT 183 ~~X 6000~~ X 10,000 = 1,830,000 Cell/

DILUTION FACTOR 1,830,000 / ~~900,000~~ 150,000 Cells/ml. = 12.2

Liters: 12.5

MEDIUM NO. LACTAL GROWTH #18

41 R/bottle 6 Pov.

PRODUCTION CONTAINER DATA AND SAMPLES — Initials: H.F.T.

1. Good	6. Nice Poor	11.	16.
2. Good	7.	12.	17.
3. Good	8.	13.	18.
4. Fair	9.	14.	19.
5. Nice Poor	10.	15.	20.

PRODUCTION SAMPLES (AND INITIALS)

To Testing 12-26-60 [illegible]

Med. Ren. 12-26-60 Lact. Growth #23 Myco #6 E Vit #3 Pen #4 CS. 17586 (5 hr. [illegible] 34°)

Held over - Slow

To Op. 3 Pov. 12-29-60 [illegible]

To Testing 1 Pov. & 5 Pov. 12-24-60 [illegible] Myco #6 [illegible]

POLIO MONOVALENT HARVEST RECORD

MONKEY NUMBERS: 10-141

POLIO MONOVALENT HARVEST NO.: 1121

DATE OF INOCULATION AND INITIALS: 12-31-60 [illegible]

DATE OF HARVEST AND INITIALS: 1-3-61 [illegible]

NUMBER OF CONTAINERS: 3 [illegible]

MEDIUM NUMBER: [illegible]

VIRUS TYPE: Type 1

SEED NUMBER: [illegible]

VOLUME:

DILUTION: 1:[illegible]

VOLUME HARVEST: 3 [illegible]

ADJUSTMENT: none

SAMPLES: [illegible] @ 25 ml [illegible]

SAMPLES		RESULTS
1 @ [illegible]	1 @ 55	
1 @ [illegible]	[illegible] @ 30	
1 @ [illegible]	1 @ 15	
	1 @ 5 [illegible]	INITIALS: [illegible]

Disposition: On 12-29-60 3 Pov. were [illegible] with Sabin [illegible] Media #5 Myco #6 @ the [illegible] of 500 ml/Pov. The virus [illegible] was [illegible] the following day 12-30-60 with an additional 500ml/Pov. On 1-3-61 Bottles [illegible] 20°C [illegible] 1-26-61 [illegible]

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Williams v. Lederle

Allan H. Hanna[illegible]

NECROPSY REPORT		NECROPSY REPORT NUMBER 577
MONKEY NUMBER JO-141 1171	SPECIES Rhesus	DATE OF EXAMINATION 12/13/60
SEX Female	WEIGHT (APPROX.) 5 pounds	DATE OF REPORT 12/13/60

Reason For Examination: (Kidney Harvest, Disease, Other)

Kidney Harvest - Production

History: (How long in Plant; Where; Symptoms; Treatment; Diagnostic Tests, Etc.)

Received in plant on 9/6/60
Isolated in B Ward Building 158 on 9/6/60
Tuberculin tested on 9/6 and 11/25 - negative
Treatments: See ward records

Primary Incision:

General Condition: **Excellent**

Visible exudates (Eyes, nose, mouth, anus, genitals) NRS

Skin NRS

Subcutis NRS

Lymph Nodes (Axillary, Inguinal) NRS

Secondary Incision:

Digestive System NRS

Oral Cavity

Esophagus

Stomach

Small intestine

Large intestine

Liver & Gall Bladder

Pancreas

Spleen

Peritoneum

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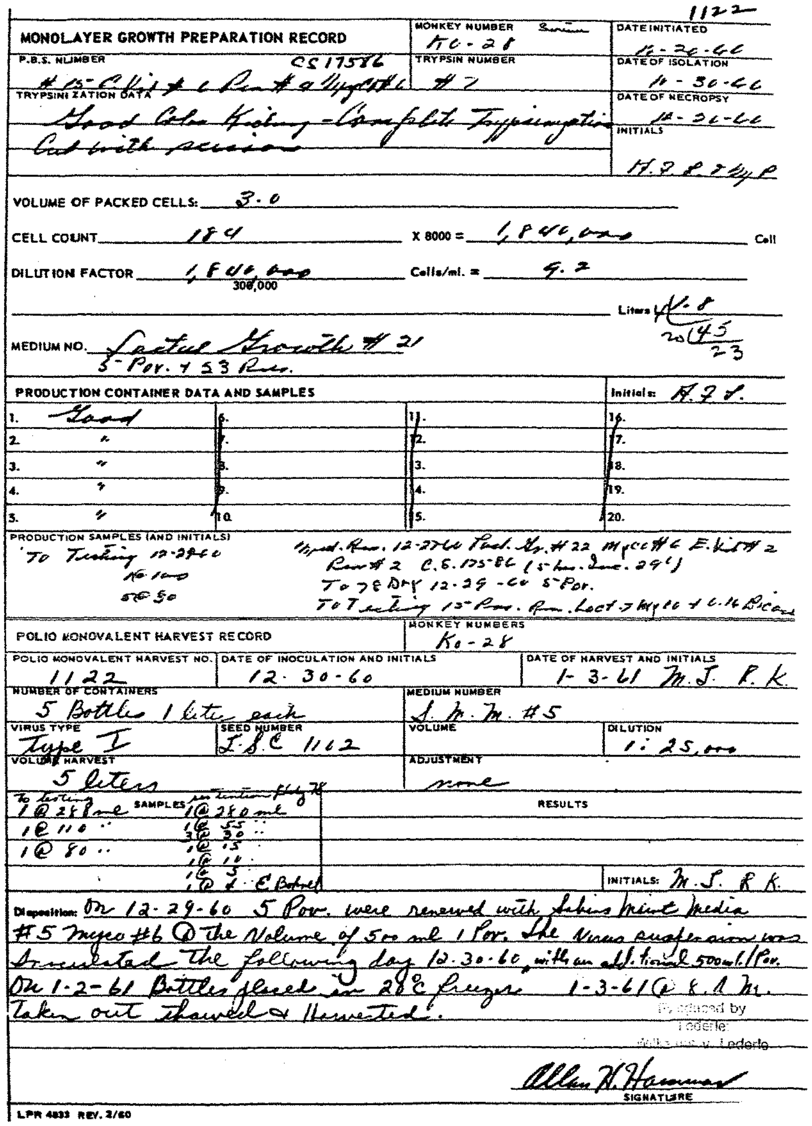
Williams v. Lederle



1122

MONOLAYER GROWTH PREPARATION RECORD

MONKEY NUMBER: Ko-28 Semien

DATE INITIATED: 12-20-60

P.B.S. NUMBER: CS 17586 #15-C Kid #6 Ren #9 Myco #6

TRYPSIN NUMBER: #7

DATE OF ISOLATION: 12-30-60

TRYPSINIZATION DATA: Good Color Kidney - Complete Trypsinization Cut with scissors

DATE OF NECROPSY: 12-20-60

INITIALS: H.J. R.F. W.P.

VOLUME OF PACKED CELLS: 3.0

CELL COUNT 184 X 8000 = 1,840,000 Cell

DILUTION FACTOR 1,840,000 / 300,000 Cells/ml. = 9.2

Liters 4.8 ... 20 / 45 / 23

MEDIUM NO. Lactal Growth # 21 5 Pov. + 53 Ren.

PRODUCTION CONTAINER DATA AND SAMPLES Initials: H.J.F.

1. Good	6.	11.	16.
2. "	7.	12.	17.
3. "	8.	13.	18.
4. "	9.	14.	19.
5. "	10.	15.	20.

PRODUCTION SAMPLES (AND INITIALS)

To Testing 12-29-60
Ko 100
5 @ 50

Myco. Ren. 12-27-60 Fluid. Gr. #22 Myco #6 E. Kid #2
Ren #2 C.S. 175-86 (5 hrs. Inc. 29°)
To 78 Dry 12-29-60 5 Pov.
To Testing 15 Pov. Ren. Lact. → Myco + 0.14 Bicarb

POLIO MONOVALENT HARVEST RECORD

MONKEY NUMBERS: Ko-28

POLIO MONOVALENT HARVEST NO.: 1122

DATE OF INOCULATION AND INITIALS: 12-30-60

DATE OF HARVEST AND INITIALS: 1-3-61 M.J. R.K.

NUMBER OF CONTAINERS: 5 Bottles 1 liter each

MEDIUM NUMBER: S.M.M. #5

VIRUS TYPE: Type I

SEED NUMBER: I.S.C 1162

VOLUME:

DILUTION: 1:25,000

VOLUME HARVEST: 5 liters

ADJUSTMENT: none

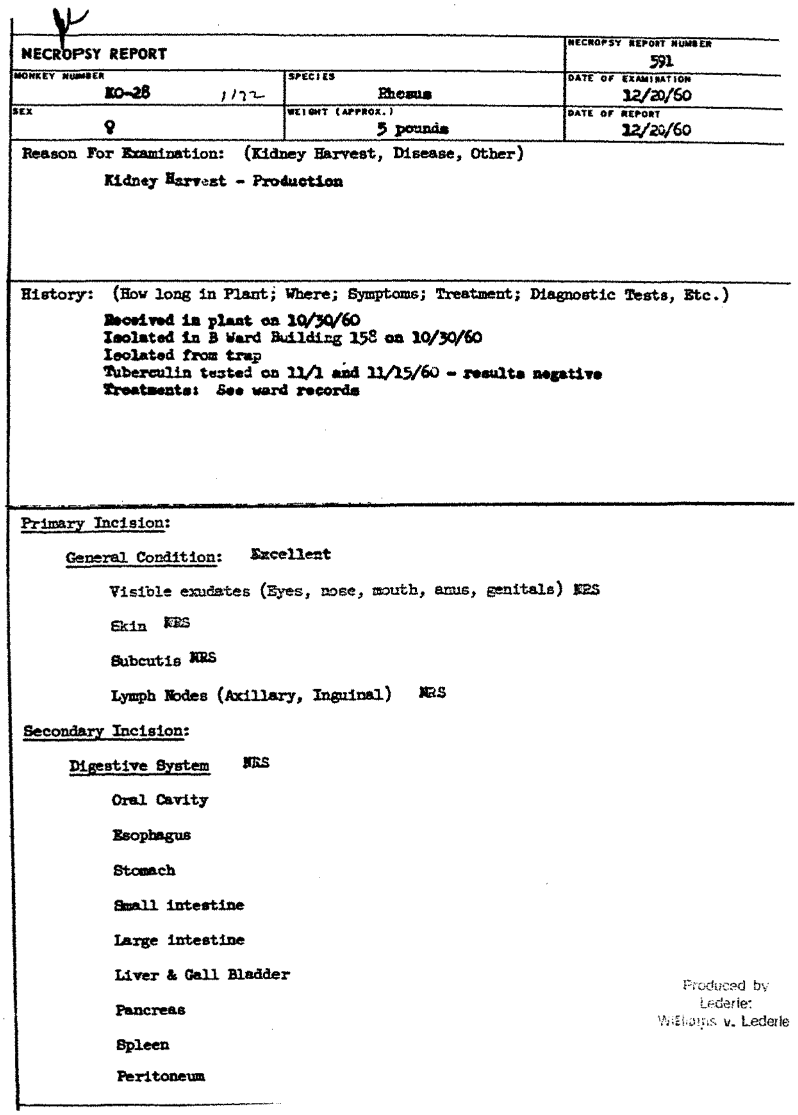
SAMPLES		RESULTS
To testing 1 @ 288 ml	for tension Fly 78 1 @ 280 ml	
1 @ 110 "	1 @ 55 " 3 @ 30 "	
1 @ 80 "	1 @ 15 1 @ 10	
	1 @ 3 1 @ 1 E Bohrel	INITIALS: M.J. R.K.

Disposition: On 12-29-60 5 Pov. were renewed with Sabins Maint Media #5 Myco #6 @ the Volume of 500 ml / Pov. The Virus suspension was Inoculated the following day 12-30-60, with an additional 500 ml / Pov. On 1-2-61 Bottles placed in 28°C freezer 1-3-61 @ 8 A.M. Taken out thawed & Harvested.

Reproduced by Lederle

Allan H. Hammar
SIGNATURE

LPR 4833 REV. 2/60



NECROPSY REPORT

MONKEY NUMBER KO-28 1172	SPECIES Rhesus	NECROPSY REPORT NUMBER 591
		DATE OF EXAMINATION 12/20/60
SEX ♀	WEIGHT (APPROX.) 5 pounds	DATE OF REPORT 12/20/60

Reason For Examination: (Kidney Harvest, Disease, Other)

Kidney Harvest - Production

History: (How long in Plant; Where; Symptoms; Treatment; Diagnostic Tests, Etc.)

Received in plant on 10/30/60
Isolated in B Ward Building 158 on 10/30/60
Isolated from trap
Tuberculin tested on 11/1 and 11/15/60 - results negative
Treatments: See ward records

Primary Incision:

General Condition: Excellent

Visible exudates (Eyes, nose, mouth, anus, genitals) NRS

Skin NRS

Subcutis NRS

Lymph Nodes (Axillary, Inguinal) NRS

Secondary Incision:

Digestive System NRS

Oral Cavity

Esophagus

Stomach

Small intestine

Large intestine

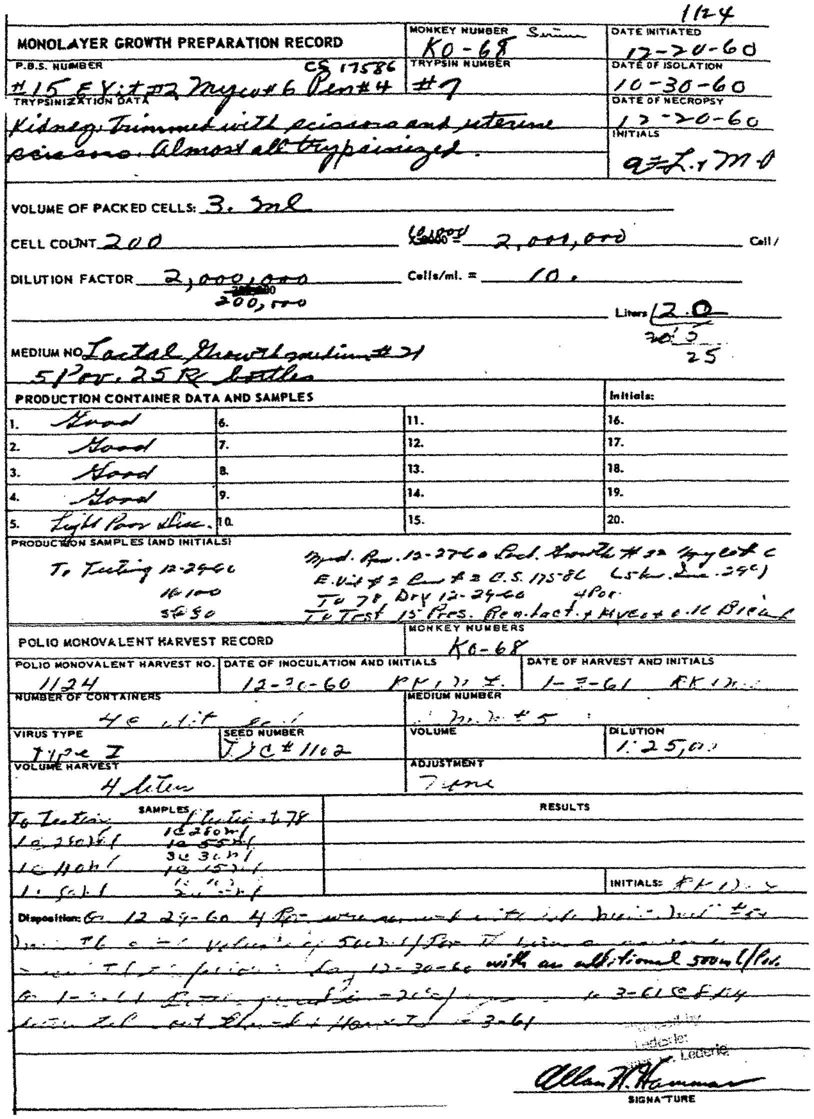
Liver & Gall Bladder

Pancreas

Spleen

Peritoneum

Produced by
Lederle:
Williams v. Lederle



1124

MONOLAYER GROWTH PREPARATION RECORD	MONKEY NUMBER Sm[illegible] K0-68	DATE INITIATED 12-20-60
P.B.S. NUMBER CS 17586 #15 E Vit #2 Myco #6 Pen #4	TRYPSIN NUMBER #7	DATE OF ISOLATION 10-30-60
TRYPSINIZATION DATA Kidney: Trimmed with scissors and uterine scissors. Almost all trypsinized.		DATE OF NECROPSY 12-20-60
		INITIALS [illegible]

VOLUME OF PACKED CELLS: 3. ml

CELL COUNT 200 [illegible] = 2,000,000 Cell/

DILUTION FACTOR 2,000,000 ~~[illegible]~~ 200,000 Cells/ml. = 10.

Liters 2.0 [illegible] 25

MEDIUM NO. Lactal Growth medium #21
5 Pov. 25 R bottles

PRODUCTION CONTAINER DATA AND SAMPLES			Initials:
1. Good	6.	11.	16.
2. Good	7.	12.	17.
3. Good	8.	13.	18.
4. Good	9.	14.	19.
5. Light Poor Disc.	10.	15.	20.

PRODUCTION SAMPLES (AND INITIALS)

To Testing 12-29-60
16-100
5-50

Med. [illegible] 12-27-60 [illegible] Growth #[illegible] Myco #[illegible]
E. Vit #2 [illegible] #2 C.S. 175-86 ([illegible] 29°)
To 78 Dry 12-29-60 4 Pov.
To Test 15 Pres. Ren. lact. + Hyec + c. 16 Bleed

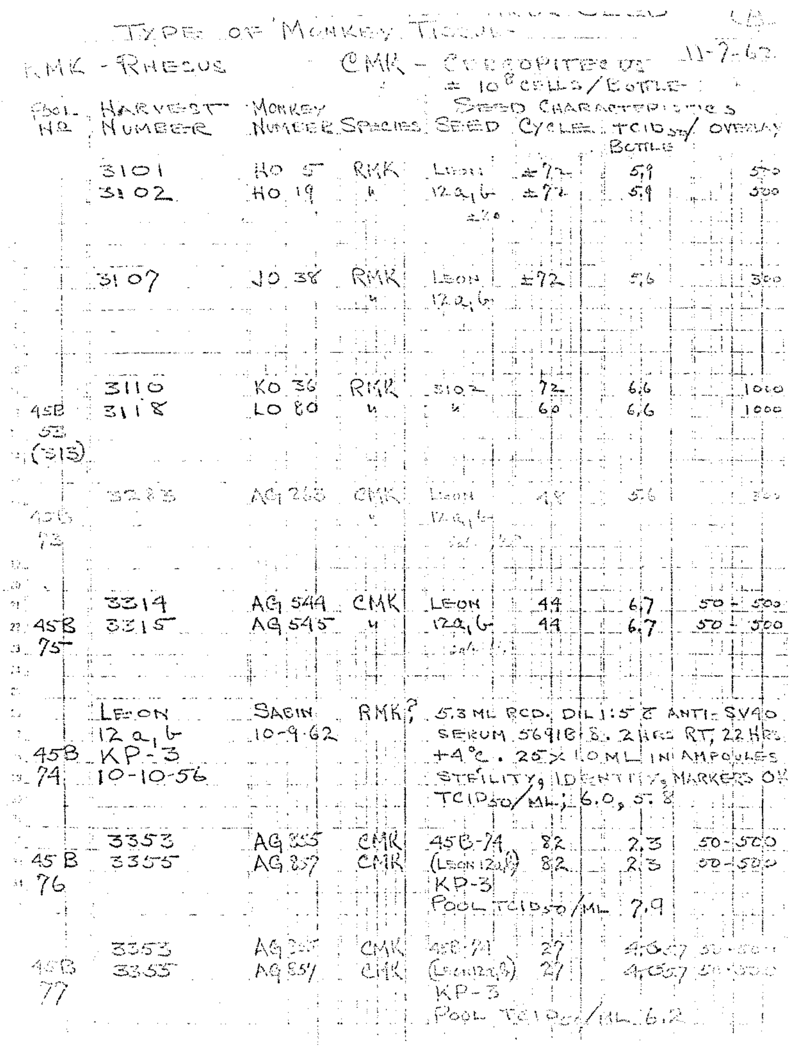
POLIO MONOVALENT HARVEST RECORD		MONKEY NUMBERS K0-68
POLIO MONOVALENT HARVEST NO. 1124	DATE OF INOCULATION AND INITIALS 12-30-60 [illegible]	DATE OF HARVEST AND INITIALS 1-3-61 [illegible]
NUMBER OF CONTAINERS 4 [illegible]		MEDIUM NUMBER [illegible] #5
VIRUS TYPE Type I	SEED NUMBER TJC #1102	VOLUME	DILUTION 1:25,0[illegible]
VOLUME HARVEST 4 liters		ADJUSTMENT [illegible]

SAMPLES		RESULTS
To Testing	[illegible] 78	
10 250 ml	10 250 ml 10 55 ml	
10 40 ml	30 30 ml 10 15 ml	
1 · 50 ml	[illegible]	INITIALS: [illegible]

Disposition: On 12-29-60 4 Pov. were [illegible] with [illegible] #[illegible]
[illegible] volume of 500 ml/Pov. [illegible]
[illegible] 12-30-60 with an additional 500 ml/Pov.
On 1-3-61 [illegible] 1-3-61 [illegible]
[illegible] Harvest [illegible] 3-61

Lederle

Allan W. Hammar
SIGNATURE



TYPE OF MONKEY TISSUE

RMK - RHESUS   CMK - CERCOPITHECUS   11-7-62

± 10^8 CELLS/BOTTLE

SEED CHARACTERISTICS

POOL NO.	HARVEST NUMBER	MONKEY NUMBER	SPECIES	SEED	CYCLE	$TCID_{50}$/ BOTTLE	OVERLAY
	3101	HO 5	RMK	LEON	±72	5,9	500
	3102	HO 19	"	12a,b	±72	5,9	500
				±70			
	3107	JO 38	RMK	LEON	±72	5,6	500
			"	12a,b			
	3110	KO 36	RMK	3102	72	6,6	1000
45B	3118	LO 80	"	"	60	6,6	1000
53							
(313)							
	3283	AG 263	CMK	LEON	48	5.6	500
45B			"	12a,b			
73							
	3314	AG 544	CMK	LEON	44	6,7	50-500
45B	3315	AG 545	"	12a,b	44	6.7	50-500
75							
	LEON	SABIN	RMK?	5.3 ML RCD. DIL 1:5 c̄ ANTI-SV40			
	12 a,b	10-9-62		SERUM 5691B 8. 2 HRS RT, 22 HRS			
45B	KP-3			+4°C. 25 × 1.0 ML IN AMPOULES			
74	10-10-56			STERILITY, IDENTITY, MARKERS OK			
				$TCID_{50}$/ML; 6.0, 5.8			
	3353	AG 855	CMK	45B-74	82	2,3	50-500
45 B	3355	AG 857	CMK	(LEON 12a,b)	82	2,3	50-500
76				KP-3			
				POOL $TCID_{50}$/ML		7.9	
	3353	AG 855	CMK	45B-74	27	4.667	50-500
45B	3355	AG 857	CMK	(LEON 12a,b)	27	4.667	50-500
77				KP-3			
				POOL $TCID_{50}$/ML		6.2	

GO 1135 REV. 11-55 PTD. IN U.S.A. 11-55

INTEROFFICE CORRESPONDENCE

Polio Vaccine Testing 8.25.61
OFFICE DATE

TO: Dr. I. Danielson ✓

XXXXXX: Dr. H. Piersma

COPY TO: Mr. S. Aiston
Dr. V. Cabasso
Mr. A. Hammar
Mrs. M. Priestley
File

SUBJECT: Poliovirus Vaccine Discards

REFERENCE:

Following is a summary of various monovalent harvests which, in our opinion, are not satisfactory for use or will not be of value to Lederle for the indicated reasons. The discard of such samples and bulk material will be necessary in order to relieve a critical storage problem. Would both of you sign your name to this memo if you agree to the proposals ? Also, you should note any alteration that you desire.

		Disposition			
		Bulk		Sample	
Harvest No.	Remarks	Hold	Disc.	Hold	Disc.
1103	Pos. VA) Make up		x		x
1105	") physical pool		x		x
1107	") #112		x		x
1108	")		x		x
1106	Pos. VA		x		x
1109	"		x		x
1114	"		x		x
1115	"		x		x
1121	Filtered PCB-2) Aliquot	x			x
1122	73% PCB Surv.) pool	x			x held
1124	71% PCB Surv.)	x			x
1125	Pos. VA		x		x
1129	Contaminated		x		x
2106	Pos. VA		x		x
2108	"		x		x
2109	"		x		x
2112	Pos. VA) Physical Pool		x		x
2120	") #212		x		x
2116	Pos. VA -Active as SIPU#17	x			x
2117	Pos. VA		x		x
2123	" FV		x		x
2126	" VA		x		x
2127	" VA & FV		x		x
2130	" VA		x		x
2135	" VA		x		x
2137	25% PCB did not survive		x		x
2143-2145	Pos. VA		x		x
2147	Pos. VA		x		x
2149	"		x		x
2133	F.U. @ PCB-1×2		x		x
2139	" " "		x		x
2140	" " "		x		x

PLAINTIFF'S EXHIBIT NO. 54
FOR IDENTIFICATION Kelleher
DATE: 10-20-? RPTR: K
PENGAD-Bayonne, N.J.

Physical Pools

101 through 105, 201 and 202, and 301 through 304.

Remarks:

RHCoggeshall
R. H. Coggeshall

J. P. Lawlis, Ph.D.

JPL:df

Remarks:

L. S. Danielson, Ph.D.

Remarks:

HDPiersma
H. D. Piersma, Ph. D.

Harvest No.	Remarks	Disposition Bulk Hold	 Disc.	 Sample Hold	 Disc.
2153	25% PCB did not survive		x		x
2157	Pos. VA		x		x
2158	"		x		x
2164	"		x		x
3103	Pos. VA } Physical		x		x
3105	" } Pool		x		x
3106	" } ~~#311~~ 312	hold	x	hold	x
3108	" }		x		x
3120	FV ≃ [illegible]		x		x
3112	Pos. VA		x		x
3113	Pos. FV		x		x
3116	" VA		x		x
3123	Pos. VA- Harvest only		x		x
3128	Pos. VA		x		x
3130	"		x		x
3131	Pos. VA & FV		x		x
3134-3137	Pos. VA		x		x
3140	Pos. VA & FV		x		x
3144	" " "		x		x
3146	Pos. FV		x		x
3149-3152	Patas Monkeys		x		x
3152	FV ≃ [illegible]		x		x
3054-3077	Lederle-Cox (Non-Modified)		x		x
3501	Pos. VA		x		x
3504	"		x		x
3507	"		x		x
3509	"		x		x
3510	"		x		x
3513-3524	25% PCB failed to survive		x		x
3526-3529	25% PCB failed to survive		x		x

Also, according to our records, the following harvests and/or pools of Lederle-Cox vaccine strains were made in Bldg. 78 for Virus Research, but they never reached the clinical trial stage, except Harvests 3022 and 3027. Thus, the following material should be discarded, if you agree:

Type I

Harvests No. 1001 through 1053.

Type II

Harvests No. 2001 through 2047.

Type III

Harvests No. 3021, 3023 through 3026, and 3029 through 3036. Also Harvests No. 3001 through 3020.

K No 25349

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

RELEASE PROTOCOL

POLIOVIRUS VACCINE, LIVE, ORAL **Type II**

Undiluted Bulk Monopool No. 2-2825

PRODUCED IN [illegible] VS LEDERLE LABS
CASE NO. 83-[illegible]
ALACHUA COUNTY, FLORIDA

K №. 25350

LEDERLE LABORATORIES
American Cyanamid Company
Pearl River, New York

CC:017/CY13-B

Date: 6/10/82

RELEASE PROTOCOL

FOR

POLIOVIRUS VACCINE, LIVE, ORAL

UNDILUTED BULK

PRODUCED [illegible] LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, [illegible]

TYPE II

MONOPOOL NO. 2-282S

K № 25351

LEDERLE LABORATORIES
American Cyanamid Company

Date: 5.5.86

POLIOVIRUS VACCINE, LIVE, ORAl
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

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II. Date of Pooling 1
III. Preservatives 1
IV. Monkey Group Records 2

LEDERLE LABS
COUNTY, FLORIDA

MANUFACTURING RECORDS

	Harvest Codes: 1 2802	2 2807	3 2810
I. Monovalent Harvest Records			
A. Production Records . . . . Kidney Cell Suspension Poliovirus Propagation	3	7	10
B. Testing Records			
1. Tissue Culture Safety Tests			
a. Pooled Fluids Taken from Production Vessels Immediately Prior to Virus Inoculation (SG)	4	8	11
b. Viral Harvest Fluids (H) . .	4,5	8	11,12
c. Pooled Fluids Taken from 25% Production Vessels at Time of Virus Harvest (PCB-1)	5	8	12
d. Pooled Fluids Taken from 25% Production Vessels 14 days after Viral Inoculation of Production Vessels (PCB-2)	5	9	12
e. Test Results on 25% Production Control Vessels (HAD)	6	9	13

LEDERLE LABORATORIES
American Cyanamid Company

K № 25352
Date: 5.5.86

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

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PRODUCE IN M[illegible] vs. LEDERLE LABS
CASE NO. 85-[illegible]
ALACHUA COUNTY, FLORIDA

	Page No. Harvest Codes: 4 2811	 5 2812	 6 2813
MANUFACTURING RECORDS			
I. Monovalent Harvest Records			
A. Production Records	14	17	20
Kidney Cell Suspension			
Poliovirus Propagation			
B. Testing Records			
1. Tissue Culture Safety Tests			
a. Pooled Fluids Taken from Production Vessels Immediately Prior to Virus Inoculation (SG)	15	18	21
b. Viral Harvest Fluids (H) . .	15	18	21
c. Pooled Fluids Taken from 25% Production Vessels at Time of Virus Harvest (PCB-1)	16	19	22
d. Pooled Fluids Taken from 25% Production Vessels 14 days after Viral Inoculation of Production Vessels (PCB-2)	16	19	22
e. Test Results on 25% Production Control Vessels (HAD)	16	19	22

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Page No.

II. Monovalent Pool Test Records

A. Unfiltered

1. Small Animal Safety Tests 23
2. M. tuberculosis (in vitro) 23
3. PPLO Test (Mycoplasma) 24,24A, 24B, 24C

B. Filtered

1. Neurovirulence Tests 25, 25A, 25B, 25C

Antibody Titrations on Sera from Monkeys used in Neurovirulence Tests 26

Potency Test on Neurovirulence Test Sample 27

NIH Reference Neurovirulence Tests 28, 28A, 28B, 28C

Antibody Titrations on Sera from Monkeys used in NIH Reference Neurovirulence Tests 29

2. rct/40 Marker Test 30

3. d-Marker Test 31

PRODUCED IN [illegible] LEDERLE LABS
CASE NO. 86-[illegible]
ALACHUA COUNTY, FLORIDA

LEDERLE LABORATORIES
American Cyanamid Company
Pearl River, N. Y.

Date: 4.4.86 Page 1

K Nº 25354

POLIOVIRUS VACCINE, LIVE, ORAL

UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

MANUFACTURING SUMMARY

I. COMPONENTS OF MONOPOOL

Harvest Code	Harvest No.	Monkey No.	Group No.	Date Filtered	Filter Type	Unfiltered Volume After Sampling
1	2802	B4-36	111	See Below	See Below	40.800 Liters
2	2807	B4-18	111			48.000 Liters
3	2810	B4-25	111			44.900 Liters
4	2811	B4-39	111			51.400 Liters
5	2812	B4-37	111			27.500 Liters
6	2813	B4-42	111			20.600 Liters

PRODUCE IN [illegible]
CASE NO. 85-1534 CA
ALACHUA COUNTY, FLORIDA

The 6 harvests were pooled in a drum, then filtered through Millipore, grade 0.22 microns.

II. DATE OF POOLING 1.14.86 VOLUME 226.8 Liters (after sampling)

III. PRESERVATIVES: None except the following antibiotics used in Poliovirus Growth Media:

Antibiotics

Streptomycin	50 mcg./ml
Neomycin	50 mcg./ml

Sterility Test: 1.15.86 Satisfactory

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LPR 6117-1 - REV. 8/82

K Nº 25355

LEDERLE LABORATORIES
American Cyanamid Company

DATE 5.12.86 PAGE 2

POLIOVIRUS VACCINE, LIVE, OR
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282

MANUFACTURING SUMMARY (CONT'D)

LEDERLE LABS
CASE NO.
ALACHUA COUNTY FLORIDA

IV. MONKEY GROUP RECORD

GROUP NO. 111

SPECIES: Rhesus

DATE ISOLATED: 2.22.84

TOTAL NO. ISOLATED: 40

EARLIEST DATE AVAILABLE FOR PRODUCTION: 4.5.84

DATE OF TB TEST: See Below

RESULT: Negative

DATE HARVESTED	MONKEY NO. IN THIS POOL	DISPOSITION	NO. REMAINING	DATE OF TB TEST	TB RESULT	HARVEST NO. IN THIS POOL	AUTOPS
4.18.84	B4-36	3 to Polio Production	37	4.13.84	Negative	2802	Negative
34	B4-18	2 to Polio Production	35	4.27.84	Negative	2807	Negative
5.9.84	B4-25 B4-39	3 to Polio Production	32	5.4.84	Negative	2810 2811	Negative
5.14.84		2 Transferred	30	4.26.84	Negative		
5.16.84	B4-37 B4-42	3 to Polio Production	27	5.11.84	Negative	2812 2813	Negative
5.23.84		2 to Polio Production	25	5.18.84	Negative		
6.26.84		24 Transferred	1	6.21.84	Negative		
9.5.84		1 Sold	0	7.27.84	Negative		

Death rate per month for the last 6 weeks: 0 %

Mary B. Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 3
K Nº 25356
POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS

I. MONOVALENT HARVEST RECORDS

HARVEST NO. 2802

A. Production Records:

Kidney Cell Suspension (Volumes Approximate)

LEDERLE LABS
ALACHUA COUNTY, FLORIDA

Rhesus Monkey No. B4-36 Total Volume: 5800 ml at 225,000 cells/ml
Volume in Production Vessels: 4300 ±ml: 43 Roller Bottles at 100 ±ml/bottle.
Volume in 25% Control Vessels: 1450 ±ml: No. 15 Type Roller at 100 ± ml/bottle.
Date Cell Growth Initiated: 4.19.84 at 36°C.
Date Cell Sheet Completed: 4.27.84 Date Used: 5.1.84
Condition of Cell Sheet at Time of Poliovirus Inoculation: Satisfactory.

Poliovirus Propagation

Poliovirus Seed Used: Lederle 45B-162 (Type II)
rle Passage No. 2 from Sabin II, P712,Ch,2ab .
42* bottles inoculated on 5.1.84 and incubated at 34± 0.5°C.
Date of cessation of growth: 5.4.84
Date Harvested: 5.4.84 Volume Harvested: 40.800 Liters (After Sampling)
Storage Temperature: -20°C

Sterility Test:

DATE ON TEST	DATE OFF TEST	Thioglycollate	Soybean-Casein Digest
		Results in Indicated Medium	
5.7.84	5.21.84	No microbial growth.	
5.7.84	5.21.84		No microbial growth.

B. Testing Records:

1. Tissue Culture Safety Tests

(For handling of test samples, and for methods of testing, see Application and Report on Manufacture of Poliovirus Vaccine, Live, Oral, Trivalent Types 1, 2 and 3, revised 11.22.76.)

*One bottle cracked - discarded.

Mary B. Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 K Nº 25357 PAGE

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2802

LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLORIDA

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Spent Growth Fluids	RMK	6181F(3)	5.23.84	0	Pass
Date Sampled: 5.1.84	CMK	3318F	6.4.84	0	Pass-Subculture
	CMK-Subc.	3315F	6.20.84	0	Pass
	RbK	19BF(2)	5.9.84	0	Invalid-Retest CPE in TCC
	RbK Retest	20AF(2)	5.30.84	0	Pass
	HuA	36	5.9.84	0	Pass
Harvest Fluids	RMK	6181F(4)	5.31.84	C	
Date Sampled: 5.4.84				C	Retest
				C	
	RMK Retest	6182F	6.22.84	0	S.C.-C
				0	Pass
				0	
	CMK	3288F	6.15.84	C	S.C.-C
				0	Retest 10 ml
				0	15 ml satisfactory
	CMK-Subc.	3318F(2)	7.6.84	C(7)	Resubculture
	CMK Resubc.	3354F	7.26.84	C(7)	Resubculture
	CMK-Resubc.	2684F(2)	8.8.84	C(7)	Retest
	CMK-Retest (10 ml)	3302F(2)	7.12.84	0	Pass-Subculture
	CMK-Subc.	3365F	8.1.84	0	Pass
	CMK-Retest	3315F(5)	10.31.85	0	
				0	Pass-Subculture
				0	Total of 35 ml satisfactory
	CMK-Subc.	3802F	12.5.85	0	Pass

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE 5

K Nº 25358

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2802

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Harvest Fluids (cont.)	RbK	19BF(3)	5.17.84	0	
Date Sampled: 5.4.84				0	
				0	
				0	
				0	Pass
	HuA	36	5.11.84	0	
				0	Pass
				0	

NOTE: S.C. in RMK, CMK and HuA satisfactory except where noted.

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
PCB-1 Fluids	RMK	6181F(3)	5.23.84	0	Pass
Date Sampled: 5.4.84	CMK	3318F	6.4.84	0	Pass-Subculture
	CMK-Subc.	3315F	6.20.84	0	Pass
	RbK	19BF(2)	5.9.84	0	Invalid-Retest CPE in TCC
	RbK-Retest	20AF(2)	5.30.84	0	Pass
	HuA	36	5.9.84	0	Pass
PCB-2 Fluids	RMK	6181F(3)	5.23.84	0D	Retest Same appearance as control
Date Sampled: 5.15.84					
	RMK-Retest	6184F(3)	3.26.85	C	Retest
	RMK-Retest	6254F(3)	12.3.85	0	Pass
	CMK	3318F	6.4.84	0	Pass-Subculture
	CMK-Subc.	3315F	6.20.84	0	Pass
	RbK	19BF(3)	5.16.84	0	Pass
	HuA	30	5.16.84	0	Pass
	BSc-1	96	5.15.84	0	Pass

PRODUCT ... LEDERLE LABS
CASE NO. 86...
ALACHUA COUNTY, FLORIDA

Mary B Ritchey

PASS
Mary B. Ritchey, Ph.D.

K Nº 25359

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 6

POLIOVIRUS VACCINE, LIVE, OR
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2802

RUBELLA TEST

DATE OF TEST	DATE OF EXAMINATION	RESULTS
5.29.84	6,1.84	Pass-No evidence of Rubella Virus

HEMADSORPTION TEST (Results on 25% Production Control Vessels)

NUMBER OF CONTAINERS	FINAL READING	SURVIVING CELL SHEETS NO.	% TOTAL	HAD RESULT
15	5.15.84	13	87 %	Negative

2 Roller Bottles contaminated - discarded.

[illegible] VS. LEDERLE LABS
CASE NO. 86-1[illegible]4 CA
ALACHUA COUNTY, FLORIDA

Mary B. Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 7
K Nº 25360
POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS

I. MONOVALENT HARVEST RECORDS

HARVEST NO. 2807

A. Production Records:

Kidney Cell Suspension (Volumes Approximate)

Rhesus Monkey No. B4-18 Total Volume: 7000 ml at 225,000 cells/ml
Volume in Production Vessels: 5200 ±ml: 52 Roller Bottles at 100 ±ml/bottle.
Volume in 25% Control Vessels: 1750 ±ml: No. 18 Type Roller at 100 ± ml/bottle.
Date Cell Growth Initiated: 5.3.84 at 36°C.
Date Cell Sheet Completed: 5.11.84 Date Used: 5.15.84
Condition of Cell Sheet at Time of Poliovirus Inoculation: Satisfactory

LEDERLE LABS
ALACHUA COUNTY, FLORIDA

Poliovirus Propagation

Poliovirus Seed Used: Lederle 45B-162 (Type II)
rle Passage No. 2 from Sabin II, P712,Ch,2ab .
51* bottles inoculated on 5.15.84 and incubated at 34± 0.5°C.
Date of cessation of growth: 5.18.84
Date Harvested: 5.18.84 ** Volume Harvested: 48.000 Liters (After Sampling)
Storage Temperature: -20°C

Sterility Test:

DATE ON TEST	DATE OFF TEST	Results in Indicated Medium: Thioglycollate	Results in Indicated Medium: Soybean-Casein Digest
5.18.84	6.1.84	No microbial growth.	
5.18.84	6.1.84		No microbial growth.

B. Testing Records:

1. Tissue Culture Safety Tests

(For handling of test samples, and for methods of testing, see Application and Report on Manufacture of Poliovirus Vaccine, Live, Oral, Trivalent Types 1, 2 and 3, revised 11.22.76.)

*One bottle cracked - discarded.
**One bottle broke - discarded.

Mary B. Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 K No. 25361 PAGE 8

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2807

ALACHUA COUNTY, FLORIDA

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Spent Growth Fluids	RMK	6181F(3)	5.23.84	0	Pass
Date Sampled: 5.15.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	SS	Invalid-Resub
				Same appearance as control.	
	CMK-Resubc.	3354F	7.25.84	C	Retest
	CMK-Retest	3315F(5)	10.31.85	0	Pass-Subculture
	CMK-Subc.	3802F	12.3.85	0	Pass
	RbK	19BF(3)	5.16.84	0	Pass
	HuA	30	5.16.84	0	Pass
Harvest Fluids	RMK	6181F(5)	6.7.84	0	
Date Sampled: 5.18.84				0	Pass
				0	
	CMK	3302F(2)	7.12.84	0	
				0	Pass-Subculture
				0	
	CMK-Subc.	3365F	8.1.84	0	Pass
	RbK	20AF	5.25.84	0	
				0	
				0	Pass
				0	
				0	
	HuA	53	5.25.84	0	
				0	Pass
				0	

NOTE: S.C. in RMK, CMK and HuA satisfactory.

PCB-1 Fluids	RMK	6181F(3)	5.23.84	0	Pass
Date Sampled: 5.18.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20AF	5.23.84	0	Pass
	HuA	56	5.23.84	0	Pass

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

K № 25362

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 9

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2807

TEST LEVEL	TISSUE	TISSUE NUMBER	DATE TESTED	FINAL READING	REMARKS
PCB-2 Fluids	RMK	6181F(4)	5.30.84	0	Pass
Date Sampled: 5.29.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	SS	Invalid-Resub. Same appearance as control. No sample available-Retest
	CMK-Retest	2684F(2)	8.7.84	0	Pass-Subculture
	CMK-Subc.	3685F(6)	10.8.85	D (7)	Retest X2
	CMK-Retest #1	3315F(5)	10.31.85	0	Pass-Subculture
	CMK-Subc.	3802F	12.3.85	0	Pass
	CMK-Retest #2	3315F(5)	10.31.85	0	Pass-Subculture
	CMK-Subc.	3802F	12.3.85	0	Pass
	RbK	20AF(2)	5.30.84	0	Pass
	HuA	57	5.30.84	0	Pass
	BSc-1	98	5.29.84	0	Pass

LEDERLE LABS
ALACHUA COUNTY, FLORIDA

RUBELLA TEST

DATE OF TEST	DATE OF EXAMINATION	RESULTS
6.12.84	6.15.84	No evidence of Rubella Virus.

HEMADSORPTION TEST

(Results on 25% Production Control Vessels)

NUMBER OF CONTAINERS	FINAL READING	SURVIVING CELL SHEETS NO.	SURVIVING CELL SHEETS % TOTAL	HAD RESULT
18	5.29.84	18	100%	Negative

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 K №. PAGE: 25363

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS

I. MONOVALENT HARVEST RECORDS

HARVEST NO. 2810

A. Production Records:

Kidney Cell Suspension (Volumes Approximate)

Rhesus Monkey No. B4-25 Total Volume: 5900 ml at 225,000 cells/ml
Volume in Production Vessels: 4400 ± ml: 44 Roller Bottles at 100 ± ml/bottle.
Volume in 25% Control Vessels: 1475 ± ml: No. 15 Type Roller at 100 ± ml/bottle.
Date Cell Growth Initiated: 5.10.84 at 36°C.
Date Cell Sheet Completed: 5.18.84 Date Used: 5.22.84
Condition of Cell Sheet at Time of Poliovirus Inoculation: Satisfactory.

[illegible] LEDERLE LABS
[illegible]
ALACHUA COUNTY, FLORIDA

Poliovirus Propagation

'ovirus Seed Used: Lederle 45B-162 (Type II)
Lederle Passage No. 2 from Sabin II, P712,Ch,2ab.
44 bottles inoculated on 5.22.84 and incubated at 34 ± 0.5°C.
Date of cessation of growth: 5.25.84
Date Harvested: 5.25.84 Volume Harvested: 44.900 Liters
(After Sampling)
Storage Temperature: -20°C

Sterility Test:

DATE ON TEST	DATE OFF TEST	Results in Indicated Medium: Thioglycollate	Results in Indicated Medium: Soybean-Casein Digest
5.31.84	6.14.84	No microbial growth.	
5.31.84	6.14.84		No microbial growth.

B. Testing Records:

1. Tissue Culture Safety Tests

(For handling of test samples, and for methods of testing, see Application and Report on Manufacture of Poliovirus Vaccine, Live, Oral, Trivalent Types 1, 2 and 3, revised 11.22.76.)

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LPR 6177-5 - REV. 5/85

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 11

K No. 25364

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2810

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Spent Growth Fluids Date Sampled: 5.22.84	RMK	6181F(3)	5.23.84	OD Same appearance as control	Retest
	RMK-Retest	6184F(3)	3.26.85	0	Pass
	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20AF	5.23.84	0	Pass
	HuA	56	5.23.84	0	Pass
Harvest Fluids Date Sampled: 5.25.84	RMK	6181F(5)	6.7.84	0 C 0	15 ml Satisfactory Retest 10 ml
	RMK-Retest (10 ml)	6182F	6.22.84	C	S.C.-C Retest
	RMK-Retest (10 ml)	6182F(4)	7.12.84	C (7)	Retest
	RMK-Retest	6180F	8.1.84	C C C	S.C.-C Retest
	RMK-Retest	6258F(11)	10.31.85	0 0 0	Pass Total 40 ml satisfactor
	CMK	3302F(2)	7.12.84	C (7) C (7) C (7)	Retest
	CMK-Retest	3354F(1)	7.26.84	C (7) C (7) C (7)	Retest
	CMK-Retest	2684F(2)	8.8.84	C (7) C (7) C (7)	Retest

LPR 4166B - 4/84

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 K Nº 25365 PAGE: 12

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2810

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Harvest Fluids (cont.) Date Sampled: 5.25.84	CMK-Retest	3315F(5)	10.31.85	0 0 0	Pass-Subculture
	CMK-Subc.	3802F	12.5.85	0	Pass
	RbK	20AF(2)	5.31.84	0 C 0 0 0	200 ml Satisfactory Retest 50 ml
	RbK- Retest (50 ml)	20BF(3)	6.22.84	0	Pass Total 250 ml satisfactory
	HuA	59	5.31.84	0 0 0	Pass

PRODUCED [illegible] LEDERLE LABS
CASE NO. 89-[illegible]
ALACHUA COUNTY, FLORIDA

NOTE: S.C. in RMK, CMK and HuA satisfactory except where noted.

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
PCB-1 Fluids Date Sampled: 5.25.84	RMK	6181F(4)	5.30.84	0	Pass
	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20AF(2)	5.30.84	0	Pass
	HuA	57	5.30.84	0	Pass
PCB-2 Fluids Date Sampled: 6.5.84	RMK	6181F(5)	6.6.84	C	Retest
	RMK-Retest	6182F	6.20.84	0	Pass
	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20BF(2)	6.13.84	0	Pass
	HuA	43F	6.6.84	0	Pass
	BSc-1	99	6.5.84	0	Pass

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

K № 25366

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 13

POLIOVIRUS VACCINE, LIVE, ORA
UNDILUTED BULK

TYPE II MONOPOOL NO.2-2825

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2810

RUBELLA TEST

DATE OF TEST	DATE OF EXAMINATION	RESULTS
6.19.84	6.22.84	No evidence of Rubella Virus

HEMADSORPTION TEST (Results on 25% Production Control Vessels)

NUMBER OF CONTAINERS	FINAL READING	SURVIVING CELL SHEETS NO.	SURVIVING CELL SHEETS % TOTAL	HAD RESULT
15	6.5.84	15	100%	Negative

[illegible] LEDERLE LABS
[illegible] CA
[illegible] COUNTY, FLORIDA

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 14
K No. 25367
POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS

I. MONOVALENT HARVEST RECORDS

HARVEST NO. 2811

A. Production Records:

Kidney Cell Suspension (Volumes Approximate)

Rhesus Monkey No. B4-39 Total Volume: 7100 ml at 225,000 cells/ml
Volume in Production Vessels: 5300 ± ml: 53 Roller Bottles at 100 ± ml/bottle.
Volume in 25% Control Vessels: 1775 ± ml: No. 18 Type Roller at 100 ± ml/bottle.
Date Cell Growth Initiated: 5.10.84 at 36°C.
Date Cell Sheet Completed: 5.18.84 Date Used: 5.22.84
Condition of Cell Sheet at Time of Poliovirus Inoculation: Satisfactory.

Poliovirus Propagation

Poliovirus Seed Used: Lederle 45B-162 (Type II)
rle Passage No. 2 from Sabin II, P712,ch,2ab.
53 bottles inoculated on 5.22.84 and incubated at 34 ± 0.5°C.
Date of cessation of growth: 5.25.84
Date Harvested: 5.25.84 Volume Harvested: 51.400 Liters
(After Sampling)
Storage Temperature: -20°C

Sterility Test:

DATE ON TEST	DATE OFF TEST	Results in Indicated Medium Thioglycollate	Soybean-Casein Digest
5.31.84	6.14.84	No microbial growth.	
5.31.84	6.14.84		No microbial growth.

B. Testing Records:

1. Tissue Culture Safety Tests

(For handling of test samples, and for methods of testing, see Application and Report on Manufacture of Poliovirus Vaccine, Live, Oral, Trivalent Types 1, 2 and 3, revised 11.22.76.)

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE 21 K N° 25374

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2813

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Spent Growth Fluids Date Sampled: 5.29.84	RMK	6181F(4)	5.30.84	0	Pass
	CMK	3388F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20AF(2)	5.30.84	0	Pass
	HuA	57	5.30.84	0	Pass
Harvest Fluids Date Sampled: 6.1.84	RMK	6181F(6)	6.14.84	0 C C	10 ml Pass Retest 15 ml
	RMK-Retest (15 ml)	6182F(3)	7.6.84	OD OD	S.C.-OD. Retest Same appearance as control.
	RMK-Retest	6254F(3)	12.5.85	0 0 0	Pass Total of 35 ml satisfactory
	CMK	3302F(2)	7.12.84	0 C 0	Retest
	CMK-Retest	3365F	8.1.84	C C C	Retest S.C.-C
	CMK-Retest	3315F(5)	10.31.85	0 0 0 0	Pass-Subculture
	CMK-Subc.	3802F	12.5.85	0	Pass
	RbK	20AF(3)	6.7.84	0 0 0 0 0	Pass
	HuA	43F	6.7.84	0 0 0	Pass

NOTE: S.C. in RMK, CMK and HuA satisfactory except where noted.

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

K № 25375

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 22

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2813

TEST LEVEL	TISSUE	TISSUE NUMBER	DATE TESTED	FINAL READING	REMARKS
PCB-1 Fluids	RMK	6181F(5)	6.6.84	0	Pass
Date Sampled: 6.1.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20BF(2)	6.13.84	0	Pass
	HuA	43F	6.6.84	0	Pass
PCB-2 Fluids	RMK	6181F(6)	6.13.84	0	Pass
Date Sampled: 6.12.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20BF(2)	6.13.84	0	Pass
	HuA	41F	6.20.84	0	Pass
	BSc-1	99	6.12.84	0	Pass

RUBELLA TEST

DATE OF TEST	DATE OF EXAMINATION	RESULTS
6.26.84	6.29.84	No evidence of Rubella Virus

HEMADSORPTION TEST

(Results on 25% Production Control Vessels)

NUMBER OF CONTAINERS	FINAL READING	SURVIVING CELL SHEETS NO.	SURVIVING CELL SHEETS % TOTAL	HAD RESULT
8	6.12.84	8	100%	Negative

PASS

Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Company

Date: 4.4.86 Page K № 25376

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS(Continued)

II. MONOVALENT POOL TEST RECORDS:

POOL NO. 2-282S

A. Unfiltered

Tests made on Pro Rata parts of unfiltered monovalent harvests listed in MANUFACTURING SUMMARY on Page 1.

1. Small Animal Safety Tests, performed as prescribed in 630.16 (a) (1),(2),(3) and (4).

Agent Tested For	Date On	Date Off	Animals Used	Per Cent Survival	Result	Remarks
B Virus	2.10.86	3.3.86	10 rabbits	100	No evidence of B virus infection	
LCM rus	2.10.86	3.24.86	5 guinea pigs	100	No evidence of TB or LCM virus	
CNS-LCM Virus	2.10.86	3.3.86	20 adult mice	100	No evidence of CNS or LCM virus infection	
Coxsackie Virus	2.10.86	2.24.86	20 suckling mice	100	No evidence of Coxsackie virus infection	20 sucklings remain for Blind Passage
Coxsackie Virus (Blind Passage)	2.24.86	3.10.86	5 suckling mice	100	No evidence of Coxsackie Virus infection	

TUBERCULOSIS (In Vitro)

DATE TESTED	QUANTITY TESTED	METHOD	INCUBATION	DATE OFF TEST	RESULTS
1.23.86	2-20 ml samples	Millipore Technic-Lowenstein-Jensen Agar	37°C	3.6.86	No Growth

H37 Ra control: Positive

Final Results: PASS

By: Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE:

K No 25368

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2811

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Spent Growth Fluids	RMK	6181F(3)	5.23.84	0	Pass
Date Sampled: 5.22.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20AF	5.23.84	0	Pass
	HuA	56	5.23.84	0	Pass
Harvest Fluids	RMK	6181F(6)	6.14.84	0	
Date Sampled: 5.25.84				0	Pass
				0	SC-C
	CMK	3288F(2)	6.15.84	0	SC-C
				0	Pass-Subculture
				0	
	CMK-Subc.	3318F(2)	7.6.84	C (7)	Resubculture
	CMK-Resubc.	3354F	7.26.84	C (7)	Resubculture
	CMK-Resubc.	2684F(2)	8.8.84	C (7)	Retest
	CMK Retest	3315F(5)	10.31.85	0	
				0	Pass-Subculture
				0	
	CMK-Subc.	3802F	12.5.85	0	Pass
	RbK	20AF(2)	5.31.84	0	
				0	
				0	Pass
				0	
				0	
	HuA	59	5.31.84	0	
				0	Pass
				0	

CASE NO.
ALACHUA COUNTY, FLORIDA
LEDERLE LABS

NOTE: S.C. in RMK, CMK and HuA satisifactory, except where noted.

Mary B. Ritchey
PASS
Mary B. Ritchey, Ph.D.

K №. 25369

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 16

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2811

TEST LEVEL	TISSUE	TISSUE NUMBER	DATE TESTED	FINAL READING	REMARKS
PCB-1 Fluids	RMK	6181F(4)	5.30.84	0	Pass
Date Sampled: 5.25.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20AF(2)	5.30.84	0	Pass
	HuA	57	5.30.84	0	Pass
PCB-2 Fluids	RMK	6181F(5)	6.6.84	0	Pass
Date Sampled: 6.5.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20BF(2)	6.13.84	0	Pass
	HuA	43F	6.6.84	0	Pass
	BSc-1	99	6.5.84	0	Pass

RUBELLA TEST

DATE OF TEST	DATE OF EXAMINATION	RESULTS
6.19.84	6.22.84	No evidence of Rubella Virus

HEMADSORPTION TEST (Results on 25% Production Control Vessels)

NUMBER OF CONTAINERS	FINAL READING	SURVIVING CELL SHEETS		HAD RESULT
		NO.	% TOTAL	
18	6.5.84	18	100%	Negative

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 17
K № 25370
POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS

I. MONOVALENT HARVEST RECORDS

HARVEST NO. 2812

A. Production Records:

Kidney Cell Suspension (Volumes Approximate)

Rhesus Monkey No. B4-37 Total Volume: 4100 ml at 225,000 cells/ml
Volume in Production Vessels: 3000 ± ml: 30 Roller Bottles at 100 ± ml/bottle.
Volume in 25% Control Vessels: 1025 ± ml: No. 11 Type Roller at 100 ± ml/bottle.
Date Cell Growth Initiated: 5.17.84 at 36°C.
Date Cell Sheet Completed: 5.25.84 Date Used: 5.29.84
Condition of Cell Sheet at Time of Poliovirus Inoculation: Satisfactory.

PRODUCED IN DISCOVERY BY LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLORIDA

Poliovirus Propagation

Poliovirus Seed Used: Lederle 45B-162 (Type II)
rle Passage No. 2 from Sabin II, P712,Ch,2ab.
30 bottles inoculated on 5.29.84 and incubated at 34 ± 0.5°C.
Date of cessation of growth: 6.1.84
Date Harvested: 6.1.84* Volume Harvested: 27.500 Liters (After Sampling)

Storage Temperature: -20°C

Sterility Test:

DATE ON TEST	DATE OFF TEST	Results in Indicated Medium Thioglycollate	Soybean-Casein Digest
6.4.84	6.18.84	No microbial growth.	
6.4.84	6.18.84		No microbial growth.

B. Testing Records:

1. Tissue Culture Safety Tests

(For handling of test samples, and for methods of testing, see Application and Report on Manufacture of Poliovirus Vaccine, Live, Oral, Trivalent Types 1, 2 and 3, revised 11.22.76.)

* One bottle contaminated - not harvested.

PASS
Mary B. Ritchey, Ph.D.

LPR 6177-5 - REV. 5/85

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE 18

K Nº 25871

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2812

TEST LEVEL	TISSUE	T.C. NUMBER	DATE TESTED	FINAL READING	REMARKS
Spent Growth Fluids Date Sampled: 5.29.84	RMK	6181F(4)	5.30.84	0	Pass
	CMK	3388F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20AF(2)	5.30.84	0	Pass
	HuA	57	5.30.84	0	Pass
Harvest Fluids Date Sampled: 6.1.84	RMK	6181F(6)	6.14.84	C C 0	5 ml satisfactory Retest 20 ml
	RMK-Retest (20 ml)	6182F(3)	7.6.84	OD OD	S.C. - OD. Same appearance as control. Retest.
	RMK-Retest	6258F(11)	10.31.85	0 0 0	Pass Total 30 ml Pass
	CMK	3302F(2)	7.12.84	0 0 0	Pass-Subculture
	CMK-Subc.	3365F	8.1.84	0	Pass
	RbK	20AF(3)	6.7.84	0 0 0 0 0	Pass
	HuA	43F	6.7.84	0 0 0	Pass

NOTE. S.C. in RMK, CMK and HuA satisfactory except where noted.

VS. LEDERLE LAB
COUNTY, FLORIDA

PASS
Mary B. Ritchey, Ph.D.

K № 25372

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 19

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TISSUE CULTURE SAFETY TESTS

HARVEST NUMBER: 2812

TEST LEVEL	TISSUE	TISSUE NUMBER	DATE TESTED	FINAL READING	REMARKS
PCB-1 Fluids	RMK	6181F(5)	6.6.84	0	Pass
Date Sampled: 6.1.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	0	Pass
	RbK	20BF(2)	6.13.84	0	Pass
	HuA	43F	6.6.84	0	Pass
PCB-2 Fluids	RMK	6181F(6)	6.13.84	0	Pass
Date Sampled: 6.12.84	CMK	3288F(2)	6.13.84	0	Pass-Subculture
	CMK-Subc.	3318F(2)	7.5.84	SS	Invalid-Resub. Same appearance as control.
	CMK-Resubc.	3354F	7.25.84	0	Pass
	RbK	20BF(2)	6.13.84	0	Pass
	HuA	41F	6.20.84	0	Pass
	BSc-1	99	6.12.84	0	Pass

RUBELLA TEST

DATE OF TEST	DATE OF EXAMINATION	RESULTS
6.26.84	6.29.84	No evidence of Rubella Virus.

HEMADSORPTION TEST (Results on 25% Production Control Vessels)

NUMBER OF CONTAINERS	FINAL READING	SURVIVING CELL SHEETS NO.	SURVIVING CELL SHEETS % TOTAL	HAD RESULT
11	6.12.84	11	100%	Negative

· PASS ·
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 20

K No 25373

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS

I. MONOVALENT HARVEST RECORDS

HARVEST NO. 2813

A. Production Records:

Kidney Cell Suspension (Volumes Approximate)

Rhesus Monkey No. B4-42 Total Volume: 2950 ml at 225,000 cells/ml
Volume in Production Vessels: 2150 ± ml: 22 Roller Bottles at 100 ± ml/bottle.
Volume in 25% Control Vessels: 738 ± ml: No. 8 Type Roller at 100 ± ml/bottle.
Date Cell Growth Initiated: 5.17.84 at 36°C.
Date Cell Sheet Completed: 5.25.84 Date Used: 5.29.84
Condition of Cell Sheet at Time of Poliovirus Inoculation: Satisfactory.

Poliovirus Propagation

Poliovirus Seed Used: Lederle 45B-162 (Type II)
rle Passage No. 2 from Sabin II,P712,Ch,2ab.
22 bottles inoculated on 5.29.84 and incubated at 34 ± 0.5°C.
Date of cessation of growth: 6.1.84
Date Harvested: 6.1.84 Volume Harvested: 20.600 Liters (After Sampling)

Storage Temperature: -20°C

Sterility Test:

DATE ON TEST	DATE OFF TEST	Results in Indicated Medium Thioglycollate	Soybean-Casein Digest
6.4.84	6.18.84	No microbial growth.	
6.4.84	6.18.84		No microbial growth.

B. Testing Records:

1. Tissue Culture Safety Tests

(For handling of test samples, and for methods of testing, see Application and Report on Manufacture of Poliovirus Vaccine, Live, Oral, Trivalent Types 1, 2 and 3, revised 11.22.76.)

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

K No 25377

LEDERLE LABORATORIES
American Cyanamid Co.
Pearl River, New York

DATE: 5.5.86 PAGE: 24

POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

TEST FOR MYCOPLASMA HYORRHINIS - FLUORESCENCE PROCEDURE

HARVEST NUMBER	VERO TISSUE	ANTI POLIO HORSE SERUM	DATE HORSE BLED	DATE SAMPLE TESTED	DATE CULTURES STAINED	RESULTS H STAIN*
2802	p.134	6088	2.21.78	6.29.84	7.2.84	Negative
2807	p.136	6088	2.21.78	8.3.84	8.6.84	Negative
2810	p.136	6088	2.21.78	8.3.84	8.6.84	Negative
2811	p.136	6088	2.21.78	8.3.84	8.6.84	Negative
2812	p.136	6088	2.21.78	9.28.84	10.1.84	Negative
2813	p.136	6088	2.21.78	9.28.84	10.1.84	Negative

LEDERLE LABS

POSITIVE CONTROLS

M-hyorrhinis (Strain 1050)	Positive
M-hyorrhinis (Strain PG29D10)	Positive
Antiserum Controls	Negative
Tissue Controls	Negative

No. of petris inoculated	6
No. of slides stained	4
Volume Sample Tested	1 ml

*Barile, M, F, Director, Mycoplasma Branch, DBP, Bureau of Biologics Protocol for DNA Staining Procedures using Bisbenzamide Fluorochrome.

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Company

Date: 4.4.86 Page 24A
K No 25378
POLIOVIRUS VACCINE,LIVE,ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS (Continued)

II. MONOVALENT POOL TEST RECORDS:

A. Unfiltered

3. Tests for Pleuropneumonia-like Organisms (PPLO-Mycoplasma)

Test made on Pool of harvests 2802,2803,2804,2805
prior to clarification, and pool of corresponding PCB-1 fluids
(pool composed of equal volume from each harvest.

Date Test Initiated 6.8.84 Dates of subcultures from broth 6.11.84 & 6.22.84

Harvests 2803,2804 and 2805 not included in Monopool 2-282S

Sample + Growth Condition	Date Read (1984)	Agar Plates 0.2 ml/plate Fab.*	Agar Plates 0.2 ml/plate Hayfl.*	Broth** 1.0 ml/tube Macroscopic Observation	Date Read (1984)	Subculture (Broth to Agar Plates) (0.2 ml/plate) Fab.	Subculture (Broth to Agar Plates) (0.2 ml/plate) Hayfl.
Aerobic 36°C							
Harvest Fluids	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
Control Fluids PCB-1	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
S-6 Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
M. pneumoniae Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
Negative Control	6.11	-	-	0/1	6.25	0/1	0/1
	6.22	0/1	0/1	0/1	7.6	0/1	0/1
Anaerobic 36°C							
Harvest Fluids	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
Control Fluids PCB-1	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
S-6 Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
M. pneumoniae Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
Negative Control	6.11	-	-	0/1	6.25	0/1	0/1
	6.22	0/1	0/1	0/1	7.6	0/1	0/1

* Fab. = Fabricant's (Swine sera)
Hayfl. = Hayflick's
** Broth = Hayflick's with 0.1% Agar

Result: PASS

By: Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Company

Date: 4.4.86 Page 24B
K No. 25379

POLIOVIRUS VACCINE,LIVE,ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS (Continued)

II. MONOVALENT POOL TEST RECORDS:

A. Unfiltered

3. Tests for Pleuropneumonia-like Organisms (PPLO-Mycoplasma)

Test made on Pool of harvests 2806, 2807, 2808, 2809 prior to clarification, and pool of corresponding PCB-1 fluids (pool composed of equal volume from each harvest.

Date Test Initiated 6.8.84 Dates of subcultures from broth 6.11.84 & 6.22.84

Harvests 2806, 2808, 2809 not included in Monopool 2-282S

Sample + Growth Condition	Date Read (1984)	Agar Plates 0.2 ml/plate Fab.*	Agar Plates 0.2 ml/plate Hayfl.*	Broth** 1.0 ml/tube Macroscopic Observation	Date Read (1984)	Subculture (Broth to Agar Plates) (0.2 ml/plate) Fab.	Subculture (Broth to Agar Plates) (0.2 ml/plate) Hayfl.
Aerobic 36°C							
Harvest Fluids	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
Control Fluids	6.11	-	-	0/2	6.25	0/2	0/2
PCB-1	6.22	0/3	0/3	0/2	7.6	0/2	0/2
S-6 Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
M. pneumoniae	6.11	-	-	2/2	6.25	2/2	2/2
Control	6.22	3/3	3/3	2/2	7.6	2/2	2/2
Negative	6.11	-	-	0/1	6.25	0/1	0/1
Control	6.22	0/1	0/1	0/1	7.6	0/1	0/1
Anaerobic 36°C							
Harvest Fluids	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
Control Fluids	6.11	-	-	0/2	6.25	0/2	0/2
PCB-1	6.22	0/3	0/3	0/2	7.6	0/2	0/2
S-6 Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
M. pneumoniae	6.11	-	-	2/2	6.25	2/2	2/2
Control	6.22	3/3	3/3	2/2	7.6	2/2	2/2
Negative	6.11	-	-	0/1	6.25	0/1	0/1
Control	6.22	0/1	0/1	0/1	7.6	0/1	0/1

* Fab. = Fabricant's (Swine sera)
Hayfl. = Hayflick's
** Broth = Hayflick's with 0.1% Agar

Result: PASS
By: Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Company

Date: 4.4.[illegible] K № 25380 Page 24C

POLIOVIRUS VACCINE,LIVE,ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS (Continued)

II. MONOVALENT POOL TEST RECORDS:

A. Unfiltered

3. Tests for Pleuropneumonia-like Organisms (PPLO-Mycoplasma)

Test made on Pool of harvests 2810,2811,2812,2813 2814 prior to clarification, and pool of corresponding PCB-1 fluids (pool composed of equal volume from each harvest.

Date Test Initiated 6.8.84 Dates of subcultures from broth 6.11.84 & 6.22.84
Harvest 2814 not included in Monopool 2-282S

Sample + Growth Condition	Date Read (1984)	Agar Plates 0.2 ml/plate Fab.*	Hayfl.*	Broth** 1.0 ml/tube Macroscopic Observation	Date Read (1984)	Subculture (Broth to Agar Plates) (0.2 ml/plate) Fab.	Hayfl.
Aerobic 36°C							
Harvest Fluids	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
Control Fluids PCB-1	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
S-6 Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
M. pneumoniae Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
Negative Control	6.11	-	-	0/1	6.25	0/1	0/1
	6.22	0/1	0/1	0/1	7.6	0/1	0/1
Anaerobic 36°C							
Harvest Fluids	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
Control Fluids PCB-1	6.11	-	-	0/2	6.25	0/2	0/2
	6.22	0/3	0/3	0/2	7.6	0/2	0/2
S-6 Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
M. pneumoniae Control	6.11	-	-	2/2	6.25	2/2	2/2
	6.22	3/3	3/3	2/2	7.6	2/2	2/2
Negative Control	6.11	-	-	0/1	6.25	0/1	0/1
	6.22	0/1	0/1	0/1	7.6	0/1	0/1

DERLE LABS

* Fab. = Fabricant's (Swine sera)
Hayfl. = Hayflick's
** Broth = Hayflick's with 0.1% Agar

Result: PASS Mary B. Ritchey
By: Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY

/PE
ROUTE

INOCULUM Poliovirus Type II PP 2-282S	PRIMARY DILUTION None	INJECTED TITER $10^{8.01}$/ml	HISTOLOGICAL EXAMINATION Dr. Thomas Davies
DATE INOCULATED 2/6/86 · DATE SACRIFICED 2/25/86	ROUTE I.T.	VOLUME 2 x 0.5 ml	NEEDLE 25g 1¼" · SPECIES Rhesus

Dilution	NUMBERS			Neg. AB Date	Paralysis	HISTOLOGICAL LESIONS OF POLIO					SCORE OF POLIO		REMARKS
	Path.	Monkey				Lumbar Cord Average	Cervical Cord Average	Brain Stem			Severity	Spread	
	W 86	Leg	Chest					Upper	Middle	Lower			
UND	0001	H1-420	M5-17	1.28.86	Negative	0	0	0	0	0	0	0	
	2	421	18	"	"	0	0	0	0	0	0	0	
	3	422	19	"	"	0	0	0	0	0*	0	0	
	4	423	22	"	"	0	0	0	0	0	0	0	
	5	424	21	"	"	0	0	0	0	0	0	0	
	6	425	20	"	"	0	0	0*	0	0	0	0	
	7	426	11	"	"	0	0	0	0	0	0	0	
	8	427	12	"	"	0	0	0	0	0	0	0	
	9	428	13	"	"	0	0	0	0	0	0	0	
	10	429	16	"	"	0	0	0	1	0	1	0	
	11	430	15	"	"	0	0	0	0	0	0	0	
	12	431	14	"	Rt.Arm Weak**	0	0	0	0	0	0	0	Cerebral Cortical Atrophy***
	13	432	7	"	Negative	0	0	0	0	0	0	0	
	14	433	6	"	"	0	0	0	0	0	0	0	
	15	434	5	"	"	0	0	0	0	0	0	0	No Track
	16	435	8	"	"	0	0	0	0	0	0	0	
	17	436	9	"	"	0	0	0	0	0	0	0	
	18	437	M5-10	"	"	0	0	0	0	0	0	0	
	19	438	A6-11	"	"	0	0	0	0	0	0	0	
	20	439	12	"	"	0	0	0	1	0	1	0	
	21	440	13	"	"	0	0	0	0	0	0	0	
	22	441	16	"	"	0	0	0	0	0*	0	0	
	23	442	15	"	"	0	0	0*	0	0	0	0	
	24	443	14	"	"	0	1	0	0	2	1	0	
	25	444	7	"	"	0	0	0	0	0	0	0	
	26	445	6	"	"	0	0	0	0	0	0	0	No Track
	27	446	5	"	"	0	0	0	0	0	0	0	
	28	447	8	"	"	0	0	0	0	0	0	0	
	29	448	9	"	"	0	0	0	0	0	0	0	
	30	449	A6-10	"	"	0	0	0	0	0	0	0	

LEGEND:
- ** – Non Progressive Paralysis
- 0* – Non Specific Lesion
- 0 – No Lesions
- 0(T) – Lesion Due to Track Only
- 1 – Minimal
- 2 – Mild
- 3 – Moderately Severe
- 4 – Severe

***localized atrophy in pre and post central cortex associated with inoculation site-

PASS

Mary B. Ritchey, Ph.D.

Mary B. Ritchey

Dilution	Significant Paralysis	Total Positive	Severity Ratio	Spread Ratio
UND	0/28	3/28	3/28	0/28
10^3				
10^4				
Totals	0/28	3/28	3/28	0/28
Decimal Equivalent	0.00	0.10	0.10	0.00
N.V. Grades	0=25	1=3	2=0	3=0	4=0

LPR 5097 · REV. 7/77

K Nº 25381

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

DAILY CLINICAL OBSERVATION

POLIOVIRUS VACCINE, LIVE, ORAL

INOCULUM: Polio PP 2-282S
TYPE: II I.T.
DATE INOCULATED: 2.6.86
DATE KILLED: 2.25.86

IL.	PATH. NO. W 86	LEG NUMBER	DAYS POST INOCULATION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	REMARKS
IND	0001	HI-420	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	2	421	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	3	422	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	4	423	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	5	424	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	6	425	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	7	426	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	8	427	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	9	428	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	10	429	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	11	430	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	12	431	*	*	*	*	*	*	*	*			*	*	*	*	*			*	*			
	13	432	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	14	433	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	15	434	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	16	435	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	17	436	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	18	437	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	19	438	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	20	439	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	21	440	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	22	441	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	23	442	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	24	443	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	25	444	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	26	445	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	27	446	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	28	447	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	29	448	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	30	449	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			

PASS
~~Mary D. Ritchey, Ph.D.~~
Mary B. Ritchey

K
Nº 25382 S
Page 25A

CODE:
1. Left leg partial paralysis
2. Left leg complete paralysis
3. Right leg partial paralysis
4. Right leg complete paralysis
5. Left arm partial paralysis
6. Left arm complete paralysis
7. Right arm partial paralysis
8. Right arm complete paralysis

*Indicates weakness only
*Right Arm Weak

LEDERLE LABS

14816A - CLINICAL RECORD OF TEST MONKEYS FOR FEDERAL REPORT - REV. 7/77

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY

1
ROUTE

INOCULUM Poliovirus Type II PP 2-282S		PRIMARY DILUTION See Below	INJECTED TITER $10^{8.01}$/ml	HISTOLOGICAL EXAMINATION Dr. Thomas Davies
DATE INOCULATED 2.6.86	DATE SACRIFICED 2.24.86	ROUTE IS	VOLUME 0.2 ml	NEEDLE 27g 3/4" — SPECIES Rhesus

Dilution	NUMBERS			Neg. AB Date	Paralysis	HISTOLOGICAL LESIONS OF POLIO					SCORE OF POLIO		REMARKS
	Path.	Monkey				Lumbar Cord Average	Cervical Cord Average	Brain Stem			Severity	Spread	
	W 86	Leg	Chest					Upper	Middle	Lower			
UND	0031	H1-450	M5-23	1.28.86	RLW(1)**	1	0	0	0	0	1	0	
	32	1	24	"	LLPP(1)**	1	1	1	2	2	1	1	
	33	2	A6- 1	"	RLPP(5)	1	1	1	1	1	1	1	
	34	3	4	"	LLPP(4)	1	0	1	0	0	1	1	
	35	4	3	"	LLCP(1)**	1	1	2	2	3	1	2	
	36	5	2	"	LLPP(1)**	1	1	1	0	1	1	1	
10^{-3}	0037	H1-456	A6-23	1.28.86	LLPP(1)**	1	0	0	0	0	1	0	
	38	7	24	"	RLPP(1)**	1	0	0	0	0	1	0	
	39	8	25	"	LLW(1)**	0	0	0	0	0	0	0	No Track
	40	9	29	"	LLW(1)**	1	0	1	1	1	1	1	
	41	60	28	"	RLW(1)**	1	0	0	0	1	1	1	
	42	1	27	"	RLW(1)**	1	1	1	1	2	1	1	
10^{-4}	0043	H1-462	A6-17	1.28.86	LLPP(1)**	0	0	0	0	0	0	0	
	44	3	18	"	LLW(1)**	1	0	0	0	0	1	0	
	45	4	19	"	LLPP(1)**	1	0	0	0	0	1	0	
	46	5	22	"	RLW(1)**	1	1	2	2	3	1	2	
	47	6	21	"	RLW(1)**	1	0	0	0	0	1	0	
	48	7	20	"	LLPP(1)**	0	0	0	0	0	0	0	

LEGEND:
- ** – Non Progressive Paralysis
- 0* – Non Specific Lesion
- 0 – No Lesions
- 0(T) – Lesion Due to Track Only
- 1 – Minimal
- 2 – Mild
- 3 – Moderately Severe
- 4 – Severe

[illegible]
Mary B. Ritchey, Ph.D.
Mary B Ritchey

Dilution	Significant Paralysis	Total Positive	Severity Ratio	Spread Ratio
UND	2/6	6/6	6/6	6/6
10^3	0/5	5/5	5/5	3/5
10^4	0/6	4/6	4/6	2/6
Totals				
Decimal Equivalent				
N.V. Grades	0=2	1=12	2=2	3=0 4=0

PR 5097 - REV. 7/77

LEDERLE LABORATORIES
Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

DAILY CLINICAL OBSERVATION

POLIOVIRUS VACCINE, LIVE, ORAL

INOCULUM: Polio Pp 2-282S	TYPE: II I.S.
DATE INOCULATED: 2.6.86	DATE KILLED: 2.24.86

	PATH. NO. W86	LEG NUMBER	DAYS POST INOCULATION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	REMARKS
D	0031	HI-450	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
	32	451	1	1	1	1	1	1	1	1			1	1	1	1	1			1				
	33	452	1*	1*	1*	1*3*	3 1*	3 1*	3 1*	3 1*			3 1*	3 1*	3 1*	3 1*	3 1*			3 1*				
	34	453	1*	1*	1*	1	1	1	1	1			1	1	1	1	1			1				
	35	454	2 3*	2 3*	2 3*	2 3*	2 3*	2 3*	2 3*	2 3*			2 3*	2 3*	2 3*	2 3*	2 3*			2 3*				
	36	455	1	1	1	1	1	1	1	1			1	1	1	1	1			1				
-3	0037	HI-456	1	1	1	1	1	1	1	1			1	1	1	1	1			1				
	38	457	3	3	3	3	3	3	3	3			3	3	3	3	3			3				
	39	458	1*	1*	1*	1*	1*	1*	1*	1*			1*	1*	1*	1*	1*			1*				
	40	459	1*	1*	1*	1*	1*	1*	1*	1*			1*	1*	1*	1*	1*			1*				
	41	460	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
	42	461	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
-4	0043	HI-462	1	1	1	1	1	1	1	1			1	1	1	1	1			1				
	44	463	1*	1*	1*	1*	1*	1*	1*	1*			1*	1*	1*	1*	1*			1*				
	45	464	1	1	1	1	1	1	1	1			1	1	1	1	1			1				
	46	465	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
	47	466	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
	48	467	1	1	1	1	1	1	1	1			1	1	1	1	1			1				

PASS
Ph.D.

ERLE LABS

CODE:
1. Left leg partial paralysis
2. Left leg complete paralysis
3. Right leg partial paralysis
4. Right leg complete paralysis
5. Left arm partial paralysis
6. Left arm complete paralysis
7. Right arm partial paralysis
8. Right arm complete paralysis

*Indicates weakness only

1816A - CLINICAL RECORD OF TEST MONKEYS FOR FEDERAL REPORT - REV. 7/77

K № 25385

LEDERLE LABORATORIES AMERICAN CYANAMID COMPANY		DATE 4.4.86	PAGE 2
		Polio Monopool II 2-282	

Results of Poliovirus antibody titrations on sera from monkeys used in Neurovirulence Test of 2.6.86

MONKEY NO.	DATE BLED	DATE TESTED	RESULT 1:4 INITIAL SERUM DILUTION	REMARKS NO. TCID$_{50}$ IN TEST DOSE TEST OF	I	II	III
M5-17	1.28.86	1.29.86	Negative	1.29.86	589	692	380
18	"	"	"				
19	"	"	"				
22	"	"	"				
21	"	"	"				
20	"	"	"				
11	"	"	"				
12	"	"	"				
13	"	"	"				
16	"	"	"				
15	"	"	"				
14	"	"	"				
7	"	"	"				
6	"	"	"				
5	"	"	"				
8	"	"	"				
9	"	"	"				
M5-10	"	"	"				
A6-11	"	"	"				
12	"	"	"				
13	"	"	"				
16	"	"	"				
15	"	"	"				
14	"	"	"				
7	"	"	"				
6	"	"	"				
5	"	"	"				
8	"	"	"				
9	"	"	"				
A6-10	"	"	"				
M5-23	"	"	"				
M5-24	"	"	"				
A6- 1	"	"	"				
4	"	"	"				
3	"	"	"				
2	"	"	"				
23	"	"	"				
24	"	"	"				
25	"	"	"				
29	"	"	"				
28	"	"	"				
27	"	"	"				
17	"	"	"				
18	"	"	"				
19	"	"	"				
22	"	"	"				
21	"	"	"				
A6-20	"	"	"				

LEDERLE LABS
ALACHUA COUNTY, FLORIDA

Mary B Ritchey
PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Company

K № 25386
Date: 4.4 86 Page 276
POLIOVIRUS VACCINE, LIVE, ORAL
UNDILUTED BULK
TYPE II MONOPOOL NO. 2-282S

POTENCY TEST ON NEUROVIRULENCE TEST SAMPLE

Titration at 36°C

Tissue Culture No. Hep_2 p. 170 Date Tested: 2.6.86

Sample Dilution*	No. Wells Positive over No. Used for Indicated Sample: Test Sample	Dilution*	NIH Ref. Virus: II, TB-3A
5.3	8/8	4.3	8/8
5.6	8/8	4.6	6/8
5.9	8/8	4.9	4/8
6.2	7/8	5.2	3/8
6.5	3/8	5.5	1/8
6.8	2/8	5.8	1/8
7.1	3/8	6.1	0/8
7.4	3/8	6.4	1/8
7.7	0/8	6.7	0/8
8.0	1/8	7.0	0/8
8.3	0/8		
8.6	0/8		
TCID 50/ml*:	8.01		6.34

PRODUCED IN MUSGROVE VS. LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLORIDA

*Expressed as log of reciprocal of dilution.

Result: Mary B Ritchey PASS
By: Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY

YPE
ROUTE

INOCULUM Poliovirus Type I NA-4 Reference	PRIMARY DILUTION	INJECTED TITER $10^{8.40}$/ml	HISTOLOGICAL EXAMINATION Dr. T. Davies
DATE INOCULATED 4.10.86 / DATE SACRIFICED 4.29.86	ROUTE I.T.	VOLUME 2 x 0.5 ml	NEEDLE 25g 1 1/4" / SPECIES Rhesus

Dilution	NUMBERS Path. W 86	Monkey Leg	Monkey Chest	Neg. AB Date	Paralysis	HISTOLOGICAL LESIONS OF POLIO Lumbar Cord Average	Cervical Cord Average	Brain Stem Upper	Brain Stem Middle	Brain Stem Lower	SCORE OF POLIO Severity	Spread	REMARKS
UND	0097	H1-516	A6- 85	4.1.86	Negative	0	0	1	0	0	1	0	NO TRACK
	8	7	86	"	"	0	0	0	0	0	0	0	
	9	8	87	"	"	0	0	0	0	0	0	0	
	100	9	90	"	"	0	0	0	0	0	0	0	
	1	20	89	"	"	0	0	0	0	0*	0	0	
	2	1	88	"	"	0	0	0	0	0	0	0	
	3	2	26	"	"	0	0	0	0	0	0	0	
	4	3	43	"	"	0	0	0	0	0	0	0	
	5	4	44	"	"	0	0	0	0	0*	0	0	
	6	5	83	"	"	0	0	0	0	0	0	0	
	7	6	63	"	"	0	0	0	0	0*	0	0	
	8	7	48	"	"	0	0	0	0	0*	0	0	
	9	8	91	"	"	0	0	0	0	0	0	0	
	10	9	92	"	"	0	0	0	0*	0	0	0	
	1	30	93	"	"	0	0	0	0	0	0	0	
	2	1	96	"	"	0	0	0	0	0	0	0	
	3	2	95	"	"	0	0	0	0	0*	0	0	
	4	3	94	"	"	0	0	0	0*	0	0	0	
	5	4	103	"	"	0	0	0	0*	0*	0	0	
	6	5	104	"	"	0	0	0	0*	0*	0	0	
	7	6	105	"	"	0	0	0	0	0	0	0	
	8	7	108	"	"	0	0	0	0	0*	0	0	
	9	8	107	"	"	0	0	0	0	0*	0	0	
	20	9	106	"	"	0	0	0	0	0	0	0	PASS
	1	40	97	"	"	0	0	0	0	0*	0	0	Mary B. Ritchey, Ph.D.
	2	1	98	"	"	0	0	0	0	0*	0	0	Mary B. Ritchey
	3	2	99	"	"	0	0	1	0*	0	1	0	
	4	3	102	"	"	0	0	0	0	0	0	0	
	5	4	101	"	"	0	0	0	1	1	1	0	
	6	5	100	"	"	0	0	0	0	0*	0	0	

LEGEND:
** – Non Progressive Paralysis
0* – Non Specific Lesion
0 – No Lesions
0(T) – Lesion Due to Track Only
1 – Minimal
2 – Mild
3 – Moderately Severe
4 – Severe

Dilution	Significant Paralysis	Total Positive	Severity Ratio	Spread Ratio
UND	0/30	3/30	3/30	0/30
10^3				
10^4				
Totals	0/30	3/30	3/30	0/30
Decimal Equivalent	.00	.10	.10	.00

VS. LEDERLE LAB

K № 25387 28

LPR 5097 - REV 7/77

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

DAILY CLINICAL OBSERVATION
POLIOVIRUS VACCINE, LIVE, ORAL

INOCULUM: Polio Ref. I NA-4
TYPE: I I.T.
DATE INOCULATED: 4.10.86
DATE KILLED: 4.29.86

DIL.	PATH. NUMBER W6	LEG NUMBER	DAYS POST INOCULATION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	REMARKS
UND	0097	H1-516	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	8	7	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	9	8	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	100	9	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	1	20	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	2	1	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	3	2	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	4	3	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	5	4	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	6	5	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	7	6	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	8	7	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	9	8	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	10	9	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	1	30	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	2	1	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	3	2	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	4	3	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	5	4	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	6	5	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	7	6	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	8	7	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	9	8	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	20	9	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	1	40	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	2	1	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	3	2	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	4	3	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	5	4	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			
	0126	HI-545	-	-	-	-	-	-	-	-			-	-	-	-	-			-	-			

Mary B. Ritchey

PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABS

K №. 25388

CODE:
1. Left leg partial paralysis
2. Left leg complete paralysis
3. Right leg partial paralysis
4. Right leg complete paralysis
5. Left arm partial paralysis
7. Right arm partial paralysis
8. Right arm complete paralysis

*Indicates weakness only

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY

TYPE
ROUTE

INOCULUM Poliovirus Type I NA-4 Reference	PRIMARY DILUTION	INJECTED TITER $10^{8.40}$/ml	HISTOLOGICAL EXAMINATION Dr. T. Davies
DATE INOCULATED 4.10.86 / DATE SACRIFICED 4.28.86	ROUTE I.S.	VOLUME 0.2 ml	NEEDLE 27g 3/4" / SPECIES Rhesus

Dilution	NUMBERS Path. W 86	Monkey Leg	Monkey Chest	Neg. AB Date	Paralysis	HISTOLOGICAL LESIONS OF POLIO Lumbar Cord Average	Cervical Cord Average	Brain Stem Upper	Brain Stem Middle	Brain Stem Lower	SCORE OF POLIO Severity	Spread	REMARKS
UND	0127	HI-546	A6-109	4.1.86	LLCP(2)	2	1	2	0	2	2	1	
	128	547	112	"	LLCP(4)	2	1	1	2	3	2	2	
	129	548	115	"	BLPP(4)	2	1	2	1	3	2	2	
	130	549	121	"	RLPP(1)**	1	1	1	1	2	1	1	
	131	550	119	"	LLCP(4)	2	1	2	1	1	2	1	
	132	551	A6-118	"	BLCP(5)	2	1	1	1	2	2	1	
10^{-3}	133	HI-552	B6- 6	4.1.86	RLPP(1)**	1	1	3	3	3	1	3	
	134	553	7	"	LLW(1)**	0	0	0	0	0	0	0	NO TRACK
	135	554	9	"	RLW(1)**	1	0	1	0	1	1	1	
	136	555	12	"	LLW(1)**	1	1	1	1	1	1	1	
	137	556	11	"	RLW(1)**	1	1	1	1	2	1	1	
	138	557	B6- 10	"	RLW(1)**	1	1	1	1	1	1	1	
10^{-4}	139	HI-558	A6-124	4.1.86	LLPP(5)	1	1	0	0	2	1	1	
	140	559	B6- 1	"	RLPP(3)	0	0	0	0	0	0	0	
	141	560	2	"	LLW(1)**	0	0	0	0	0	0	0	
	142	561	5	"	LLW(1)**	1	1	1	0	2	1	1	
	143	562	4	"	RLW(1)**	0	0	0	0	0	0	0	
	0144	563	B6- 3	"	RLPP(6)	2	1	2	1	2	2	2	

LEGEND:
** – Non Progressive Paralysis
0* – Non Specific Lesion
0 – No Lesions
0(T)– Lesion Due to Track Only
1 – Minimal
2 – Mild
3 – Moderately Severe
4 – Severe

Mary B Ritchey PASS
Mary B. Ritchey, Ph.D.

Dilution	Significant Paralysis	Total Positive	Severity Ratio	Spread Ratio
UND	5/6	6/6	11/6	8/6
10^3	0/5	5/5	5/5	7/5
10^4	3/6	3/6	4/6	4/6

Totals
Decimal Equivalent
N.V. Grades 0=3 1=7

PR 5097 - REV. 7/77

K № 25389
28B

LEDERLE LABORATORIES
A Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

DAILY CLINICAL OBSERVATION
POLIOVIRUS VACCINE, LIVE, ORAL

INOCULUM: Polio Ref. I NA-4
TYPE: I I.S.
DATE INOCULATED: 4.10.86
DATE KILLED: 4.28.86

IL.	PATH. NUMBER W6	LEG NUMBER	DAYS POST INOCULATION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	REMARKS
IND	0127	H1-546	1*	2	2	2 3*	2 3*	2 3*	2 3*	2 3*			2 3*	2 3*	2 3*	2 3*	2 3*			2 3*				
	8	7	1*	1 3*	1 3*	2 3*	2 3*	2 3*	2 3*	2 3*			2 3*	2 3*	2 3*	2 3*	2 3*			2 3*				
	9	8	1*	1	1	1 3	1 3	1 3	1 3	1 3			1 3	1 3	1 3	1 3	1 3			1 3				
	30	9	3	3 1*	3 1*	3 1*	3 1*	3 1*	3 1*	3 1*			3 1*	3 1*	3 1*	3 1*	3 1*			3 1*				
	1	50	1*	1*	1	2	2	2 3*	2 3	2 3			2 3	2 3	2 3	2 3	2 3			2 3				
	0132	1	3	4 1*	4 1*	4 1	4 2	4 2	4 2	4 2			4 2	4 2	4 2	4 2	4 2			4 2				
0^{-3}	0133	H1-552	3	3	3	3	3	3	3	3			3	3	3	3	3			3				
	4	3	1*	1*	1*	1*	1*	1*	1*	1*			1*	1*	1*	1*	1*			1*				
	5	4	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
	6	5	1*	1*	1*	1*	1*	1*	1*	1*			1*	1*	1*	1*	1*			1*				
	7	6	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
	0138	7	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
		—																						
0^{-4}	0139	H1-558	1*	1*	1*	1*	1	1	1	1			1	1	1	1	1			1				
	40	9	3*	3*	3	3	3	3	3	3			3	3	3	3	3			3				
	1	60	1*	1*	1*	1*	1*	1*	1*	1*			1*	1*	1*	1*	1*			1*				
	2	1	1*	1*	1*	1*	1*	1*	1*	1*			1*	1*	1*	1*	1*			1*				
	3	2	3*	3*	3*	3*	3*	3*	3*	3*			3*	3*	3*	3*	3*			3*				
	0144	H1-563	3*	3*	3*	3*	3*	3	3	3			3	3	3	3	3			3				

PASS
Mary B. Ritchey, Ph.D.

LEDERLE LABS

K № 25390

28C

CODE:
1. Left leg partial paralysis
2. Left leg complete paralysis
3. Right leg partial paralysis
4. Right leg complete paralysis
5. Left arm partial paralysis
6. Left arm complete paralysis
7. Right arm partial paralysis
8. Right arm complete paralysis

*Indicates weakness only

K №. 25391

LEDERLE LABORATORIES AMERICAN CYANAMID COMPANY		DATE 6.6.86 — PAGE 29 Polio Reference I, NA-4

PRODUCE [illegible] LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLORIDA

Results of Poliovirus antibody titrations on sera from monkeys used in Neurovirulence Test of 4-10-86

MONKEY NO.	DATE BLED	DATE TESTED	RESULT 1:4 INITIAL SERUM DILUTION	REMARKS NO. TCID50 IN TEST DOSE TEST OF	I	II	III
A6-85	4-1-86	4-2-86	negative	4-2-86	251	562	562
86	"	"	"				
87	"	"	"				
90	"	"	"				
89	"	"	"				
88	"	"	"				
26	"	"	"				
43	"	"	"				
44	"	"	"				
83	"	"	"				
63	"	"	"				
48	"	"	"				
91	"	"	"				
92	"	"	"				
93	"	"	"				
96	"	"	"				
95	"	"	"				
94	"	"	"				
103	"	"	"				
104	"	"	"				
105	"	"	"				
108	"	"	"				
107	"	"	"				
106	"	"	"				
97	"	"	"				
98	"	"	"				
99	"	"	"				
102	"	"	"				
101	"	"	"				
100	"	"	"				
109	"	"	"				
112	"	"	"				
115	"	"	"				
121	"	"	"				
119	"	"	"				
A6-118	"	"	"				
B6-6	"	"	"				
7	"	"	"				
9	"	"	"				
12	"	"	"				
11	"	"	"				
B6-10	"	"	"				
A6-124	"	"	"				
B6-1	"	"	"				
2	"	"	"				
5	"	"	"				
4	"	"	"				
B6-3	"	"	"				

Mary B Ritchey
PASS
Mary B. Ritchey Ph D

LEDERLE LABORATORIES
American Cyanamid Company
Pearl River, New York

Date: 4.4.86 Page 30

POLIOVIRUS VACCINE, LIVE, ORAL K No. 25202

UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS (Cont'd)

II. MONOVALENT POOL TEST RECORDS:

B. Filtered

2. rct/40 Marker Test 0.2 ml inoculated per tube.

REPRODUCE IN [illegible] VS LEDERLE LABS [illegible] COUNTY

Date Tested: 2.20.86 TC Used: RMK 6327 TC Controls: Satisfactory

Virus Dilution	No. Tubes Positive over No. Used for Indicated Sample and Temperature					
	Sample		NIH Ref.I, Mahoney		Lederle Ref. II #1	
	36°C	40°C	36°C	40°C	36°C	40°C
10^{-0}		0/8				0/8
10^{-1}		0/8				0/8
10^{-2}		0/8				0/8
$10^{-4.0}$					10/10	
$10^{-4.5}$					7/10	
$10^{-5.0}$			8/8	8/8	3/10	
$10^{-5.5}$	10/10				1/10	
$10^{-6.0}$	9/10		8/8	8/8	0/10	
$10^{-6.5}$	6/10					
$10^{-7.0}$	4/10		8/8	8/8		
$10^{-7.5}$	2/10					
$10^{-8.0}$			3/8	2/8		
$10^{-9.0}$			0/8	0/8		
$TCID_{50}$/ml:	7.2	< 0.7	8.5	8.4	5.5	< 0.7

(Reported as log to the base 10 of the reciprocal of the dilution)

36°-40° (rct/40) > 6.5

Seed rct/40(36°-40°) > 6.2 (45B-162 tested 4.24.79)

Control rct/40(36°-40°) > 4.8

Result: Mary B. Ritchey PASS

By: Mary B. Ritchey, Ph.D.

LEDERLE LABORATORIES
American Cyanamid Company

K Nº 25393

Date: 4.4.86 Page: 31

POLIOVIRUS VACCINE, LIVE, ORAL

UNDILUTED BULK

TYPE II MONOPOOL NO. 2-282S

MANUFACTURING RECORDS (Cont'd)

II. MONOVALENT POOL TEST RECORDS

B. Filtered

3. d-Marker Test 0.2 ml inoculated per bottle.

Date Tested: 2.21.86 TC Used: RMK 6327 TC Controls: Satisfactory

MUSGROVE VS LEDERLE LABS
ALACHUA COUNTY, FLORIDA

Log Virus Dilution	Test Sample		NIH Reference (Mahoney) HEp-2		Lederle Ref. II #1	
	Acid	Alkaline	Acid	Alkaline	Acid	Alkaline
Undiluted	0,0				0,0	
1	0,0				0,0	
2	0,0				0,0	
3						
3.5						15,12
4						
4.5						1,1
5		34,36				
6		2,6	20, 21	35,28		
7			2,4	2,1		
8						
PFU/ml	<0.7	7.3	8.1	8.1	<0.7	5.3
EOP:	>6.6		0		> 4.6	

Ratio EOP of Sample to EOP of Seed: 1.3 (45B-162 tested 5.18.79 EOP: > 5.2

Ratio EOP of Sample to EOP of Reference Type II#1 : 1.4

Result: PASS
By: Mary B. Ritchey, Ph.D.

GLOSSARY OF ABBREVIATIONS USED IN RELEASE PROTOCOLS

AB	Antibody or Antibodies
Alk	Alkaline pH - generally reflected in cell degeneration
BAF	Bovine Amniotic Fluid
BME	Basal Medium (Eagle)
BSc-1	Bureau of Standards, Cercopithecus-1, Monkey Cell Strain
Blake	Glass Culture Bottle, 150 cm^2 surface area
BSS	Balanced Salt Solution
B Virus	Cercopithecid Herpesvirus 1
C	Non-viral Contamination
CF	Complement Fixation
CFL	Confluent, referring to plaques
CFR	Code of Federal Regulations
CGMP	Current Good Manufacturing Practice
CMK	Cercopithecus Monkey Kidney Tissue (Primary)
CMV	Cytomegalovirus
CNS	Central Nervous System
CPE	Cytopathic Effect
CPF 150	Corning Plastic Flask, 150 cm^2 surface area
CS	Calf Serum
Ctr	Container
D or Degen	Non-specific Degeneration
DBS	Division of Biologics Standards
DBV	Diluted Bulk Vaccine
DFA	Direct Fluorescent Antibody Staining Technique

PRODUCE IN MUSGROVE VS. LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLA

Diln	Dilution
d marker	"delayed" plaque genetic marker test
Eala	Earles lactalbumin hydrolysate medium
EOP	Efficiency of Plating
exam	examination
F	Frozen. Refers to outgrowth of cell suspension frozen at -196°C.
FA	Fluorescent Antibody
FCS	Fetal Calf Serum
FFC	Final Filled Container - Final Vaccine
FRhL	Fetal Rhesus Lung Cell Strain
FSD	Final Serum Dilution
FV	Foamy Virus
H	Viral Harvest Fluids
H Stain	DNA Staining Procedure for Mycoplasma Detection using Bisbenzamide Fluorochrome
HAD	Hemadsorption
Hep-2	Human Epithelial Carcinoma Cell Line
HuA	Human Amnion Tissue Culture
IF	Immunofluorescence
IM	Intramuscular
Inoc.	Inoculated or Inoculum
Inv.	Invalid Test, followed by reason for invalidation.
IP	Intraperitoneal
IS	Intraspinal
ISD	Initial Serum Dilution
IT	Intrathalamic

PRODUCE IN [illegible] VS. LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLORIDA

LCM	Lymphocytic Choriomeningitis
Led 130	Lederle 130, Diploid Human Lung Fibroblast Cell Strain
LN	Liquid Nitrogen
MEM-E	Minimal Essential Medium - Eagle
MF	Millipore® Filtered
MIT	Metabolic Inhibition Test
ml	milliliter
MP	Monopool, synonym for Physical Pool
MT	Microtitration
NA	Not Applicable
neg.	Negative
NIH	National Institutes of Health
NV	Neurovirulence
0	Negative for Cytopathology
OBRR	Office of Biologics Research & Review
OPV	Oral Poliovirus Vaccine
p.	Passage, refers to cell culture passage, followed by the passage number
Path.	Pathology
PCB	Production Control Bottles
PCB-1	Fluids pooled from Production Control Bottles at time of viral harvests from production vessels.
PCB-2	Fluids pooled from Production Control Bottles 14 days after viral inoculation of production vessels.
PCF	Plastic Culture Flask, 75 cm^2 surface area
PE	Production Eligible, refers to freezer bank monkeys.
PFU	Plaque Forming Units
POV	Povitsky Bottle
PP	Physical Pool, synonym for Monopool

PRODUCED IN [illegible] VS. LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLORIDA

PPLO	Pleuropneumonia-like Organism
PV	Poliovirus
RbK	Rabbit Kidney Tissue Culture (Primary)
RC	Round Cell Cytopathology
RCT/40	Reproductive Capacity Temperature 40°. Genetic Marker Test
Ref.	Reference
Resubc.	Resubculture
Ret.	Retest or Retested
Rk-13	Rabbit Kidney Epithelial-type cell line obtained from H. Hopps, Division of Biological Standards
SC	Serum Control
SCD	Soybean Casein Digest Sterility Test Medium
SG	Spent Growth Fluids. Fluids pooled from Production Vessels immediately prior to virus inoculation.
SOP	Standard Operating Procedure
SS	Sheet Sloughed
Subc.	Subculture or Subcultured
Sub Q	Subcutaneous
SV40	Simian Virus 40, Vacuolating Agent
TB	Mycobacterium Tuberculosis
TC	Tissue Culture
TCC	Tissue Culture Control
TCID50	Tissue Culture Infectious Dose, 50% End Point
Thio	Thioglycollate Sterility Test Medium
TNTC	Too Numerous to Count, referring to plaques or colonies
TV	Trivalent Vaccine
Und	Undiluted
Key (3)	Number in parenthesis following tissue culture number indicates shipment of culture prepared from freezer bank.

PRODUCE IN [illegible] VS. LEDERLE LABS
CASE NO. [illegible]
ALACHUA COUNTY, FLORIDA

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Attorneys for Defendants Wyeth, American Cyanamid Company and
Lederle Laboratories, a Division of American Cyanamid Company

DAVID RIVARD, as Administrator of the Estate of LINDSAY M. RIVARD, deceased, and DAVID RIVARD and DIANE RIVARD as the parents and natural guardians of LINDSAY M. RIVARD, Deceased, Plaintiffs,	SUPERIOR COURT OF NEW JERSEY LAW DIVISION: BERGEN COUNTY DOCKET NO. BER-L-8470-01 Civil Action
v.	
AMERICAN HOME PRODUCTS, INC., AMERICAN CYANAMID COMPANY, LEDERLE LABORATORIES, a division of AMERICAN CYANAMID, JOHN DOE ONE THROUGH ONE-HUNDRED (1-100), Defendants.	**DEFENDANTS AMERICAN CYANAMID AND LEDERLE LABORATORIES' SUPPLEMENTAL RESPONSES TO PLAINTIFFS' REQUESTS FOR ADMISSIONS**

Defendants American Cyanamid and Lederle Laboratories (collectively "American Cyanamid") hereby provide the following supplemental response to Plaintiffs' Request for Admissions:

REQUEST FOR ADMISSION NO. 1:

American Cyanamid has no records showing that it tested SOM Type I for monkey neurovirulence.

RESPONSE:

This Request seeks information regarding neurovirulence testing and is

therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist reflecting its own testing on the SOM Type I material itself and will provide a supplemental response to this Request in the event that information is located that will permit it to admit or deny this Request. American Cyanamid also states that it has conducted extensive monkey neurovirulence testing on monopools of Type I poliovirus vaccine material that were derived from the SOM Type I strain, the results of which demonstrate the acceptability of SOM Type I for use in making vaccine.

REQUEST FOR ADMISSION NO. 2:

American Cyanamid has no records showing that it tested SOM Type II for monkey neurovirulence.

RESPONSE:

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist reflecting its own testing on the SOM Type II material itself and will provide a supplemental response to this Request in the event that information is located that will permit it to admit or deny this Request. American Cyanamid also states that it has conducted extensive monkey neurovirulence testing on monopools of Type II poliovirus vaccine material that were derived from the SOM Type II strain, the results of which demonstrate the acceptability of SOM Type II for use in making vaccine.

REQUEST FOR ADMISSION NO. 3:

American Cyanamid has no records showing that it tested SOM Type III for monkey neurovirulence.

RESPONSE:

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist reflecting its own testing on the SOM Type III material itself and will provide a supplemental response to this Request in the event that information is located that will permit it to admit or deny this Request. American Cyanamid also states that it has conducted extensive monkey neurovirulence testing on monopools of Type III poliovirus vaccine material that were derived from the SOM Type III strain, the results of which demonstrate the acceptability of SOM Type III for use in making vaccine.

REQUEST FOR ADMISSION NO. 5:

American Cyanamid has no record of submitting to the government the test results on the following seed material for adventitious agents, 3101, 3107, 3102, 1101, 2107, 45B73, 45B74, 701S, 801S, 1102, 45B 164, 45B51, 45B52, 45B53, prior to their use in the manufacture of either additional seeds and/or monovalent pools and submitted to the United States of America.

RESPONSE:

American Cyanamid objects to this Request because it seeks information regarding materials not used in the vaccine identified by Plaintiffs as the vaccine administered to Lindsay Rivard and because American Cyanamid was not required to submit test results to the government with respect to these materials. Notwithstanding this, American Cyanamid denies the Request as stated, and states that the vaccines produced from these materials were tested for adventitious agents, and the protocols for these lots were submitted to the government and approved for release prior to their use in vaccine.

REQUEST FOR ADMISSION NO. 7:

American Cyanamid did not utilize a subculture test of the CMK tissue culture material when it tested the following seeds: 45B51, 45B52, 45B77, 45B85, 45B76, 45B53, 3101, 3102, 3107, 1101, 1102, 2107.

RESPONSE:

American Cyanamid objects to this Request because it seeks information

regarding materials not used in the vaccine identified by Plaintiffs as the vaccine administered to Lindsay Rivard. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether the specified tests were performed and will provide a supplemental response to this Request in the event that information is located that will permit it to admit or deny this Request.

REQUEST FOR ADMISSION NO. 8:

American Cyanamid's seeds 45B51, 45B52, 45B53, 3101, 3102, 3107, 2107, Seed 1001 and 1102 were produced in Rhesus monkey kidney tissue and not in African Green monkey kidney tissue.

RESPONSE:

American Cyanamid objects to this Request because it seeks information regarding materials not used in the vaccine identified by Plaintiffs as the vaccine administered to Lindsay Rivard. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine the species of monkeys used to manufacture these materials and will provide a supplemental response to this Request in the event that information is located that will permit it to admit or deny this Request.

REQUEST FOR ADMISSION NO. 9:

Seeds 701 S and 801 S were not tested for monkey neurovirulence prior to their use as intermediate seed material to produce Type I seed 45B 160 and Type II seed 45B 162.

RESPONSE:

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid admits that intermediate materials 701S and 801S were not tested for monkey neurovirulence and that there was no requirement that these intermediate materials be tested for neurovirulence. American Cyanamid further states that seeds 45B160 and 45B162, which were produced from 701S and 801S, as well as every one of the monopools that were

made from these seeds, were tested for monkey neurovirulence, and that all of those test results were submitted to the United States government. In addition, the government also tested most or all of these monopools for monkey neurovirulence and specifically determined that all of the test results for released monopools satisfied the existing regulatory requirements for monkey neurovirulence testing and thus issued an official release for each such monopool before any was used in vaccine production.

REQUEST FOR ADMISSION NO. 10:

Seed 45B164 was not tested by American Cyanamid for neurovirulence before its use in producing seed 45B165.

RESPONSE:

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid admits that 45B164 was not tested for monkey neurovirulence and that there was no requirement that this material be tested for neurovirulence. American Cyanamid further states that seed 45B165, which was produced from 45B164, as well as all of the monopools made from this seed, were tested for monkey neurovirulence, and that all of those test results were submitted to the United States government. In addition, the government also tested most or all of these monopools for monkey neurovirulence and specifically determined that all of the test results for released monopools satisfied the existing regulatory requirements for monkey neurovirulence testing and thus issued an official release for each such monopool before any was used in vaccine production.

REQUEST FOR ADMISSION NO. 11:

Sabin Original strain of Type I was not tested for neurovirulence by defendant.

RESPONSE:

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Moreover, American Cyanamid states that, based on historical records, it does not appear that American Cyanamid ever even used Sabin Original strain material for the manufacture of its Type I poliovirus vaccine. Nonetheless,

American Cyanamid admits that it never tested the Sabin Original strain of Type I ("SO Type I") for monkey neurovirulence and states that there was no requirement that this material be tested for neurovirulence by American Cyanamid.

__REQUEST FOR ADMISSION NO. 12:__

The Sabin Original strain of Type II was not tested for neurovirulence by defendant.

__RESPONSE__

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Moreover, American Cyanamid states that, based on historical records, it does not appear that American Cyanamid ever even used Sabin Original strain material for the manufacture of its Type II poliovirus vaccine. Nonetheless, American Cyanamid admits that it never tested the Sabin Original strain of Type II ("SO Type II") for monkey neurovirulence and states that there was no requirement that this material be tested for neurovirulence by American Cyanamid.

__REQUEST FOR ADMISSION NO. 13:__

Sabin Original strain of Type III was not tested for neurovirulence by defendant.

__RESPONSE:__

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence as this case concerns Plaintiffs' claim that SV40 was in the polio vaccine allegedly administered to Lindsay Rivard and caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information currently known or readily obtainable is insufficient to enable it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist that would enable it to admit or deny whether it tested the Sabin Original strain of Type III ("SO Type III") for monkey neurovirulence and will provide a supplemental response to this Request in the event that such information becomes available. American Cyanamid further states that there was no requirement that this material be tested for neurovirulence by American Cyanamid and that all of the monopools ultimately made from material derived from SO Type III were tested for monkey neurovirulence and the test

results were submitted to the United States government. The government also tested most or all of these monopools for monkey neurovirulence and determined that all of the test results on released monopools satisfied the existing regulatory requirements for monkey neurovirulence testing and thus issued an official release for each such monopool before any was used in vaccine production.

REQUEST FOR ADMISSION NO. 16:

American Cyanamid did not test Sabin Original Merck Type III to determine whether or not adventitious agents were present.

RESPONSE:

Denied.

REQUEST FOR ADMISSION NO. 17:

American Cyanamid did not test Sabin Original Strain material Type I, for potency before its use.

RESPONSE:

American Cyanamid objected to this Request as it seeks information regarding potency that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the potency of the vaccine. Moreover, even if potency were relevant here, American Cyanamid states that, based on historical records, it does not appear that American Cyanamid ever even used Sabin Original strain material for the manufacture of its Type I poliovirus vaccine. Nonetheless, American Cyanamid admits that it never tested SO Type I for potency. In addition, there was no requirement that this material be tested by American Cyanamid for potency.

REQUEST FOR ADMISSION NO. 18:

American Cyanamid did not test Sabin Original Strain material Type II, for potency before its use.

RESPONSE:

American Cyanamid objects to this Request as it seeks information

regarding potency that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the potency of the vaccine. Moreover, even if potency were relevant here, American Cyanamid states that, based on historical records, it does not appear that American Cyanamid ever even used Sabin Original strain material for the manufacture of its Type II poliovirus vaccine. Nonetheless, American Cyanamid admits that it never tested SO Type II for potency and further states that there was no requirement that this material be tested by American Cyanamid for potency.

REQUEST FOR ADMISSION NO. 19:

American Cyanamid did not test Sabin Original Strain material Type III, for potency before its use.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding potency that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the potency of the vaccine. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist that would enable it to determine whether it tested SO Type III for potency and will provide a supplemental response to this Request in the even that information becomes available that would permit it to admit or deny this Request. American Cyanamid further states that there was no requirement that this material be tested by American Cyanamid for potency, that all of the harvests and monopools ultimately made from material derived from SO Type III were tested for potency, that the test results were submitted to the United States government for approval, and that all monopools ever used to make vaccine released in the United States were specifically approved for such use by the United States government.

REQUEST FOR ADMISSION NO. 20:

American Cyanamid did not test Sabin Original Merck Type I, for potency before its use.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding potency that is neither relevant nor reasonably calculated to lead to the

discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the potency of the vaccine. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist that would enable it to determine whether it tested SOM Type I for potency and will provide a supplemental response to this Request in the event that information becomes available to it that would enable it to admit or deny this Request. American Cyanamid further states that there was no requirement that this material be tested by American Cyanamid for potency, that all of the harvests and monopools ultimately made from material derived from SOM Type I were tested for potency, that the test results were submitted to the United States government for approval, and that all monopools ever used to make vaccine released in the United States were specifically approved for such use by the United States government.

REQUEST FOR ADMISSION NO. 21:

American Cyanamid did not test Sabin Original Merck Type II, for potency before its use.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding potency that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the potency of the vaccine. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist that would enable it to determine whether SOM Type II was tested for potency and will provide a supplemental response to this Request in the event that information becomes available that would permit it to admit or deny this Request. American Cyanamid further states that there was no requirement that this material be tested by American Cyanamid for potency, that all of the harvests and monopools ultimately made from material derived from SOM Type II were tested for potency, that the test results were submitted to the United States government for approval, and that all monopools ever used to make vaccine released in the United States were specifically approved for such use by the United States government.

REQUEST FOR ADMISSION NO. 22:

American Cyanamid did not test Sabin Original Merck Type III, for potency before its use.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding potency that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves plaintiffs' claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the potency of the vaccine. Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist that would enable it to determine whether SOM Type III was tested for potency and will provide a supplemental response to this Request in the event that information becomes available that would permit it to admit or deny this Request. American Cyanamid further states that there was no requirement that this material be tested by American Cyanamid for potency, that all of the harvests and monopools ultimately made from material derived from SOM Type III were tested for potency, that the test results were submitted to the United States government for approval, and that all monopools ever used to make vaccine released in the United States were specifically approved for such use by the United States government.

REQUEST FOR ADMISSION NO. 23:

American Cyanamid did not test the Sabin Original Strain material Type I for the marker tests identified in the regulations which were applicable to Oral poliovirus vaccine.

RESPONSE:

American Cyanamid objects to this Request as it is incomprehensible and therefore unanswerable as it does not understand what it means to "test . . . for the marker tests." To the extent that marker tests are tests related to neurovirulence or identity, American Cyanamid further objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the neurovirulence or identity of the vaccine. American Cyanamid further objects to this Request on the ground that it does not identify "any marker tests identified in the regulations which were applicable to Oral poliovirus vaccine" that would apply to "Strain material."

American Cyanamid further states that, even if "marker tests" were relevant here, it does not appear that American Cyanamid ever even used SO Type I to manufacture its Type I poliovirus vaccine. Nonetheless, American Cyanamid admits that it never performed marker tests on SO Type I, denies that there were

any such marker tests identified in the regulations that applied to "Strain material" and states that there was no requirement in the regulations to perform such tests.

REQUEST FOR ADMISSION NO. 24:

American Cyanamid did not test the Sabin Original Strain material Type II for the marker tests identified in the regulations which were applicable to Oral poliovirus vaccine.

RESPONSE:

American Cyanamid objects to this Request as it is incomprehensible and therefore unanswerable as it does not understand what it means to "test . . . for the marker tests." To the extent that marker tests are tests related to neurovirulence or identity, American Cyanamid further objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the neurovirulence or identity of the vaccine. American Cyanamid further objects to this Request on the ground that it does not identify "any marker tests identified in the regulations which were applicable to Oral poliovirus vaccine" that would apply to "Strain material."

American Cyanamid further states that, even if "marker tests" were relevant here, it does not appear that American Cyanamid ever even used SO Type II to manufacture its Type II poliovirus vaccine. **Nonetheless, American Cyanamid admits that it never performed marker tests on SO Type II, denies that there were any such marker tests identified in the regulations that applied to "Strain material" and states that there was no requirement in the regulations to perform such tests.**

REQUEST FOR ADMISSION NO. 25:

American Cyanamid did not test the Sabin Original Strain material Type III for the marker tests identified in the regulations which were applicable to Oral poliovirus vaccine.

RESPONSE:

American Cyanamid objects to this Request as it is incomprehensible and therefore unanswerable as it does not understand what it means to "test . . . for the marker tests." To the extent that marker tests are tests related to neurovirulence or identity, American Cyanamid further objects to this Request as it seeks information

that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the neurovirulence or identity of the vaccine. American Cyanamid further objects to this Request on the ground that it does not identify "any marker tests identified in the regulations which were applicable to Oral poliovirus vaccine" that would apply to "Strain material."

Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine whether records exist that would enable it to determine whether it performed marker tests on SO Type III and will provide a supplemental response to this Request in the event that information becomes available that would permit it to admit or deny the Request. American Cyanamid further states that there was no requirement that marker tests be performed on this material by American Cyanamid, that the required marker tests were performed on all of the monopools ultimately made from material derived from SO Type III, that the test results were submitted to the United States government for approval, and that no monopools made from material derived from SO Type III were used in the manufacture of polio vaccine released for use in the United States without the specific approval of the United States government.

REQUEST FOR ADMISSION NO. 26:

American Cyanamid did not test the Sabin Original Merck material Type I for the marker tests identified in the regulations which were applicable to Oral poliovirus vaccine, and submit the results to the Government prior to licensure.

RESPONSE:

American Cyanamid objects to this Request as it does not understand what it means to "test . . . for the marker tests." To the extent that marker tests are tests related to neurovirulence or identity, American Cyanamid further objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the neurovirulence or identity of the vaccine. American Cyanamid further objects to this Request on the ground that it does not identify "any marker tests identified in the regulations which were applicable to Oral poliovirus vaccine" that would apply to "Strain material."

Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information known or readily obtainable is insufficient to enable it to admit or deny this request, which relates to events that presumably occurred at the time of licensure more than forty years ago. American Cyanamid states that it did perform marker tests on SOM Type I but that it is unaware of whether these results were sent to the government. American Cyanamid further states that there was no requirement that marker tests be performed by American Cyanamid on this material, that the required marker tests were performed on all of the monopools ultimately made from material derived from SOM Type I, that the test results were submitted to the United States government for approval, and that no monopools made from material derived from SOM Type I were used in the manufacture of polio vaccine released for use in the United States without the specific approval of the United States government.

REQUEST FOR ADMISSION NO. 27:

American Cyanamid did not test the Sabin Original Merck material Type II for the marker tests identified in the regulations which were applicable to Oral poliovirus vaccine, and submit the results to the Government prior to licensure.

RESPONSE:

American Cyanamid objects to this Request as it does not understand what it means to "test . . . for the marker tests." To the extent that marker tests are tests related to neurovirulence or identity, American Cyanamid further objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the neurovirulence or identity of the vaccine. American Cyanamid further objects to this Request on the ground that it does not identify "any marker tests identified in the regulations which were applicable to Oral poliovirus vaccine" that would apply to "Strain material."

Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information known or readily obtainable is insufficient to enable it to admit or deny this request, which relates to events that presumably occurred at the time of licensure more than forty years ago. American Cyanamid states that it did perform marker tests on SOM Type II but that it is unaware of whether these results were sent to the government. American Cyanamid further states that there was no requirement that marker tests be performed by American Cyanamid on this material, that the required marker tests were performed on all of the monopools ultimately made from material derived from SOM Type II, that the test results were submitted to the United States government for approval, and that no monopools

made from material derived from SOM Type II were used in the manufacture of polio vaccine released for use in the United States without the specific approval of the United States government.

REQUEST FOR ADMISSION NO. 28:

American Cyanamid did not test the Sabin Original Merck material Type III for the marker tests identified in the regulations which were applicable to Oral poliovirus vaccine, and submit the results to the Government prior to licensure.

RESPONSE:

American Cyanamid objects to this Request as it can not understand what it means to "test . . . for the marker tests." To the extent that marker tests are tests related to neurovirulence or identity, American Cyanamid further objected to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that the polio vaccine contained SV40 and that SV40 can cause cancer. It does not involve issues related to the neurovirulence or identity of the vaccine. American Cyanamid further objected to this Request on the ground that it does not identify "any marker tests identified in the regulations which were applicable to Oral poliovirus vaccine" that would apply to "Strain material."

Notwithstanding this, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to review documents to whether such tests were performed and will provide a supplemental response to this Request in the event that information is obtained that will permit American Cyanamid to admit or deny this Request. American Cyanamid further states that there was no requirement that marker tests be performed by American Cyanamid on this material, that the required marker tests were performed on all of the monopools ultimately made from material derived from SOM Type III, that the test results were submitted to the United States government for approval, and that no monopools made from material derived from SOM Type III were used in the manufacture of polio vaccine released for use in the United States without the specific approval of the United States government.

REQUEST FOR ADMISSION NO. 37:

American Cyanamid claimed to have neutralized Sabin Original Merck Type III, but has no test results indicating that following the neutralization, there was an absence of SV40 in the Sabin Original Merck Type III.

RESPONSE:

American Cyanamid objects to this Request as overly broad and unduly burdensome and because it seeks information that is neither relevant nor reasonably calculated to lead to discovery of admissible evidence. SOM Type III strain provided to American Cyanamid in 1960 by Dr. Albert Sabin and/or Merck was not used in the vaccine identified as the vaccine administered to Lindsay Rivard. Notwithstanding this, American Cyanamid denies this Request. Existing historical records show that rabbit anti-serum was used to neutralize SOM Type III, that all harvest subsequently made with SOM Type III were tested using CMK and other tissue cultures, that no released harvest showed results that suggested that SOM Type III was contaminated with SV40 and that all harvest test results manufactured from SOM Type III that were negative for cytopathic effect demonstrate the absence of SV40 in SOM Type III.

REQUEST FOR ADMISSION NO. 38:

Prior to May 1991, the maximum amount of tissue culture passages was five for the Orimune product.

RESPONSE:

Although Plaintiffs moved to compel an answer to this request, the motion was frivolous as American Cyanamid had fully responded, and no further response is required.

REQUEST FOR ADMISSION NO. 42:

At least one of the harvests utilized in Seed 45B51 did not meet the requirements for tissue culture safety testing, but nevertheless was used to make the product.

RESPONSE:

American Cyanamid objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. The seed identified was not used in the vaccine allegedly administered to Lindsay Rivard. Notwithstanding this, American Cyanamid denies that any harvest used to make 45B51 did not meet the "requirements for tissue culture safety testing," as all products used to make all vaccine ever approved by the FDA for distribution and administration in the United States met all requirements established by the FDA for tissue culture safety testing and all other mandatory regulatory requirements.

REQUEST FOR ADMISSION NO. 44:

American Cyanamid has not identified monovalent pools 111, 112, 211, 212, 311 and 312 to any litigant who has been involved in polio vaccine litigation involving the Orimune product.

RESPONSE:

American Cyanamid objects to this Request as vague, ambiguous, and unintelligible. American Cyanamid still does not understand what this Request means and therefore can neither admit nor deny it.

REQUEST FOR ADMISSION NO. 53:

Sabin Seed LSc-2ab SOM was the only source utilized to produce Type I monovalent pool number 116.

RESPONSE:

American Cyanamid objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. The monopool identified in this Request was not used in the vaccine identified as the vaccine administered to Lindsay Rivard. Nonetheless, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine the origin of Type I monopool 116 and will provide a supplemental response to this Request in the event that information is located that will permit it to admit or deny this Request.

REQUEST FOR ADMISSION NO. 54:

Seed numbers 3102 and 3107 were utilized to produce Type III monovalent pool number 314.

RESPONSE:

American Cyanamid objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. The monopool identified in this Request was not used in the vaccine identified as the vaccine administered to Lindsay Rivard. Nonetheless, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine the origin of Type III monopool 314 and will provide a supplemental response to this Request in the event that

information is located that will permit it to admit or deny this Request.

REQUEST FOR ADMISSION NO. 55:

Sabin seed P712 ch 2ab (SOM) was the sole source of seed material used to produce Type II monovalent pool number 213.

RESPONSE:

American Cyanamid objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. The monopool identified in this Request was not used in the vaccine identified as the vaccine administered to Lindsay Rivard. Nonetheless, American Cyanamid states that, after reasonable inquiry, the information presently known or readily obtainable is insufficient to permit it to admit or deny this Request, but that it is continuing to engage in a search to determine the origin of Type II monopool 213 and will provide a supplemental response to this Request in the event that information is located that will permit it to admit or deny this Request.

REQUEST FOR ADMISSION NO. 56:

Between November 28, 1961 and January 1, 1999, American Cyanamid's test on the reference vaccine NA2, NA3 and NA4 did not yield a final grade 3, or final grade 4 score on the intrathalamic test for either severity or spread.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding neurovirulence testing, which is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. The case involves claims that SV40 was in the polio vaccine and that it caused cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid cannot admit or deny this Request as stated, as the answer depends on what a "final" grade 3 or "final" grade 4 is. Moreover, responding to this Request would require American Cyanamid to review all the tests performed on hundreds of monkeys from 1961 to 1999 with respect to an issue that has no conceivable relevance to this litigation. American Cyanamid can state that the government's test on the reference vaccine had dozens of monkeys that scored grade 3 and grade 4 on the IT test for severity or spread or both in the 1960s, 1970s, and 1980s. All the documents with American Cyanamid's test scores and the government's test scores on IT and IS have been produced in Previous Litigation, and Plaintiffs are as easily able to review these thousands of documents as American Cyanamid is. Finally, American Cyanamid states that all test results on monkey neurovirulence tests were provided to the United States government and that no monopools were used in the manufacture of polio vaccine released for use in the United States without the specific approval of the United States government as

satisfying all then-existing regulatory requirements.

REQUEST FOR ADMISSION NO. 57:

Between November 28, 1961 and January 1, 1999, when American Cyanamid conducted the intrathalamic test on the Reference vaccine NA2, NA3 and NA4 no monkeys were paralyzed from the vaccine under test.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding neurovirulence testing, which is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that SV40 was in the polio vaccine and that it caused cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid is unable to admit or deny this Request. Responding to this Request would require American Cyanamid to review all the intrathalamic (IT) tests performed on hundreds of monkeys from 1961 to 1999 with respect to an issue that has no conceivable relevance to this litigation. All the documents with the results of the IT test have been produced in Previous Litigation, and Plaintiffs are as easily able to review these thousands of documents as American Cyanamid is. Finally, American Cyanamid states that all test results on monkey neurovirulence tests were provided to the United States government and that no monopools were used in the manufacture of polio vaccine released for use in the United States without the specific approval of the United States government as satisfying all then-existing regulatory requirements.

REQUEST FOR ADMISSION NO. 58:

Between November 28, 1961 and January 1, 1999, when American Cyanamid conducted its intraspinal test, no single monkey had a score of 3 severity and 3 spread and no monkey had scored a final grade 4 on either the severity or spread when conducting the test on the reference vaccine NA2, NA3 and NA4 by American Cyanamid.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding neurovirulence testing, which is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that SV40 was in the polio vaccine and that it caused cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid denies this request. American Cyanamid states that it had monkeys with a score of 3 spread and 3 severity in the

1960s and as late as 1980 had a monkey with a score of 3 spread and 2 severity. In addition, to respond fully to this Request would require American Cyanamid to review all of the intraspinal tests performed on thousands of monkeys from 1961 to 1999 with respect to an issue that has no conceivable relevance to this litigation. All the documents with the results of the IS test have been produced in Previous Litigation, and Plaintiffs are as easily able to review these thousands of documents as American Cyanamid is. Finally, American Cyanamid states that all test results on monkey neurovirulence tests were provided to the United States government and that no monopools were used in the manufacture of polio vaccine released for use in the United States without the specific approval of the United States government as satisfying all then-existing regulatory requirements.

REQUEST FOR ADMISSION NO. 59:

American Cyanamid was informed of the test results of any of the monovalent pools tested by the United States of America if American Cyanamid requested the said information from the United States of America and its regulatory agencies.

RESPONSE:

American Cyanamid objects to this Request as incomprehensible and unintelligible and therefore unanswerable. American Cyanamid further states that contrary to plaintiffs' motion to compel, it did not object to this Request on relevance grounds. American Cyanamid still does not understand what this Request means and therefore can neither admit nor deny it as written. American Cyanamid admits that at some point it became aware of some monkey neurovirulence test results performed by the United States on some monopools (at times because it requested the information) but that the United States did not routinely provide American Cyanamid with the government's test results. In addition, American Cyanamid has no knowledge of what the United States would have given it (if anything) in addition to what it asked for. Nor can American Cyanamid determine what it either asked for or what the United States gave it for the forty-year time span covered by this irrelevant request.

REQUEST FOR ADMISSION NO. 61:

Orimune vaccine is no longer sold in the United States of America to physicians for the administration of polio vaccine to their patients.

RESPONSE:

American Cyanamid states that although Plaintiffs moved to compel a further answer on this request, the motion in regards to this Request was frivolous as American Cyanamid fully responded to this Request, and no further response is

required.

REQUEST FOR ADMISSION NO. 62:

American Cyanamid began its effort to produce inactivated polio vaccine in the early 1980's.

RESPONSE:

American Cyanamid objects to this Request as it is entirely irrelevant, because this case has nothing whatsoever to do with IPV. Nonetheless, American Cyanamid admits that it began research and development efforts with respect to developing an inactivated polio vaccine (IPV) during the early 1980s but that the development work was never completed and that American Cyanamid was never licensed to produce IPV.

REQUEST FOR ADMISSION NO. 64:

American Cyanamid prepared

(a) daily

(b) weekly

(c) monthly

(d) quarterly

(e) semi-annual

(f) annual reports

of adverse reactions from the Oral Polio Vaccine.

RESPONSE:

American Cyanamid states that although Plaintiffs moved to compel a further answer on this request, the motion in regards to this Request was frivolous as American Cyanamid responded to this Request, and no further response is required.

REQUEST FOR ADMISSION NO. 65:

American Cyanamid conducted field trials in over 2,000,000 people for its Orimune prior to licensure.

RESPONSE:

American Cyanamid objects to this Request as it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. Notwithstanding this, American Cyanamid admits that field trials were conducted in which oral polio vaccine (OPV) made from the Sabin strains was administered to millions of people prior to the time that it and other manufacturers were licensed to make OPV from these strains.

REQUEST FOR ADMISSION NO. 68:

American Cyanamid did not perform neurovirulence tests on the following seeds:

(a) 45B74

(b) 45B77

(c) 45B53

(d) 701 S

(e) 45B157

(f) 45B158

(g) 801 S

(h) 45B 164

(i) LSc 2ab KP2

(j) LSc 2ab KP3

(k) P712 ch1 2ab-KP2

(l) P712 ch1 2ab-KP3.

RESPONSE:

This Request seeks information regarding neurovirulence testing and is therefore neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that SV40 was in the polio vaccine

allegedly administered to Lindsay Rivard and that it caused her cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Notwithstanding this, American Cyanamid is currently engaged in a search to determine whether such a test was performed on these materials and will provide a supplemental response to this Request as soon as the information becomes available. American Cyanamid further states that neurovirulence tests were not required on all of the materials listed. In addition, all of the monopools made from these materials or other material derived from them were tested for monkey neurovirulence and the test results were submitted to the United States government for approval, and approval by the United States was specifically obtained before use of any monopools in the manufacture of polio vaccine released in the United States.

REQUEST FOR ADMISSION NO. 74:

Defendant has no record of receiving official approval for the use of seed 3102.

RESPONSE:

American Cyanamid objects to this Request as overly broad and unduly burdensome and because it seeks information that is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. The seed identified in this Request was not used in the vaccine identified as the vaccine administered to Lindsay Rivard. Notwithstanding this, American Cyanamid states that it assumes "official approval" means FDA/DBS approval, and if that is correct, American Cyanamid denies this Request. While American Cyanamid is unaware of a document specifically "approving" this material, the FDA/DBS would issue such approval by approving the release of the five consecutive monopools manufactured from this material.

REQUEST FOR ADMISSION NO. 75:

Defendant has no record of receiving official approval for the use of seed 3107.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 76:

Defendant has no record of receiving official approval for the use of seed 45B76.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 77:

Defendant has no record of receiving official approval for the use of seed 45B77.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 78:

Defendant has no record of receiving official approval for the use of seed 45B85.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 79:

Defendant has no record of receiving official approval for the use of seed 45B164.

RESPONSE:

American Cyanamid states that although Plaintiffs moved to compel a further answer on this request, the motion regarding this Request is frivolous as American Cyanamid did not object, and no further response is required.

REQUEST FOR ADMISSION NO. 80:

Defendant has no record of receiving official approval for the use of seed 45B53.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 81:

Defendant has no record of receiving official approval for the use of seed 2107.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 82:

Defendant has no record of receiving official approval for the use of seed 1102.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 83:

Defendant has no record of receiving official approval for the use of seed 1101.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 84:

Defendant has no record of receiving official approval for the use of seed 45B51.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 85:

Defendant has no record of receiving official approval for the use of seed 45B52.

RESPONSE:

See Response to Request for Admission No. 74.

REQUEST FOR ADMISSION NO. 88:

On or about November 25, 1977 the United States of America made a determination that the number of Lederle lots which would have exceeded the experimental limits for the neurovirulence safety test in a retrospective analysis was the following: (a) type III from seeds 3101, 3102, 3107, all 13 monovalent pools; (b) Type III from seed 45B76 23 out of 35 monovalent pools; (c) Type III from seed 45B77 9 out of 14 monovalent pools; (d) Type III from seed 45B85 24 out of 36 monovalent pools.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding neurovirulence testing, which is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that SV40 was in the polio vaccine and that it caused cancer. It is not a case involving paralytic polio, in which neurovirulence of the vaccine might be relevant. Further, American Cyanamid objected to this interrogatory as unintelligible and therefore unanswerable.

Notwithstanding this, American Cyanamid admits that it is aware of a memorandum dated on or about November 25, 1977, that references some of the subjects listed in the Request but that the Request does not accurately describe what the memorandum states and that the memorandum speaks for itself. American

Cyanamid thus denies this Request.

REQUEST FOR ADMISSION NO. 89:

Prior to the advent of I.P.V. and O.P.V., most of the paralytic cases of polio in the United States were from Type I wild virus.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding paralytic polio, which is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that SV40 was in the polio vaccine and that it caused cancer. It is not a case involving paralytic polio. American Cyanamid also states that it has no way of ascertaining the answer to this Request, which does not reference any defined period of time prior to the licensure of IPV in the 1950s. American Cyanamid is aware that epidemiological data collected in various medical journals and publications by agencies such as the CDC contain information relevant to this subject, but even those data would not enable one to answer this Request due to its vagueness.

REQUEST FOR ADMISSION NO. 90:

Prior to Orimune being licensed for use in the United States, the rate of paralytic poliomyelitis from the wild polio virus had dropped in 1961 to less than 1000 cases per year according to the Poliomyelitis Surveillance Unit, Epidemiology Branch of the Communicable Disease Center and of that amount only 778 had paralysis lasting more than 60 days.

RESPONSE:

American Cyanamid objects to this Request as it seeks information regarding paralytic polio, which is neither relevant nor reasonably calculated to lead to the discovery of admissible evidence. This case involves claims that SV40 was in the polio vaccine and that it caused cancer. It is not a case involving paralytic polio. Notwithstanding this, American Cyanamid states that it has no factual knowledge about this Request, and Plaintiffs provide no source for the quoted data. Even if Plaintiffs had provided a source, all American Cyanamid could do would be to admit (or deny) that the quoted source did (or did not) say what Plaintiffs claim it said. To the extent that the Request is suggesting a disagreement with the decision made by all public health agencies in the United States in the 1960s to switch from IPV to OPV (a policy that Plaintiffs' principal counsel plainly disagrees with) or a disagreement with the wisdom of that decision to maintain OPV as the vaccine of choice for the 1960s, 1970s, 1980s, and most of the 1990s, i.e., until OPV had wiped out wild polio in the Western Hemisphere, then American Cyanamid denies the Request, because even according to this Request there were still 1000 cases of wild polio per year. Plaintiffs may think that "only 778" cases of permanent paralysis per year from wild poliovirus is a small number, but that number was reduced to zero by OPV.

REQUEST FOR ADMISSION NO. 91:

American Cyanamid conducted an intramuscular test on Seed 45B51 and two monkies W61/2854 and W61/2860 with final neurovirulence grade 4.

RESPONSE:

Because American Cyanamid objects to this Request on the ground that it is, among other things, utterly unintelligible, it cannot admit or deny the Request. If an answer is required, American Cyanamid denies this Request because it cannot admit something that it does not understand.

REQUEST FOR ADMISSION NO. 92:

The test results of the intramuscular examination on seed 45B51 as conducted by American Cyanamid was not submitted to the United States of America by American Cyanamid with its license application and prior to its use of the seed as a production seed for the Orimune product.

RESPONSE:

American Cyanamid states that although Plaintiffs moved to compel a further answer on this request, the motion in regards to this Request is frivolous as American Cyanamid answered this Request, and no further response is required.

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LEDERLE LABORATORIES

A Division of AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK 10965
AREA CODE 914 732-5000

January 15, 1990

Paul Parkman, M.D., Director
Center for Biologics Evaluation and Research
ATTN: HFB-500
Building 29
8800 Rockville Pike
Bethesda, Maryland 20892

Dear Doctor Parkman:

Lederle Laboratories wishes to formally request the release of a number of Types 1, 2 and 3 Sabin Polio Monopools which were produced in Rhesus primary monkey kidney cells. As you are aware, the Lederle product license for Polio Vaccine Live Oral Trivalent provides for the production of poliovirus in Macaca, Cercopethecus or Patas monkeys. Furthermore, live oral polio vaccine was once routinely produced in Macaca primary monkey kidney cell cultures. The Rhesus monkeys used to prepare these monopools were all domestic-bred in either the Texas or Louisiana Lederle-controlled monkey colonies.

These Rhesus monopools were manufactured between September 1984 and April 1987 and samples and testing protocols were supplied to CBER for release for further manufacturing between December 1985 and October 1987. These monopools represent several million doses of trivalent oral polio vaccine.

Monopool Number	Protocol Sent to CBER
Type 1 - 263	11/11/86
Type 1 - 265	7/30/87
Type 2 - 283	6/17/86
Type 3 - 501	12/5/85
Type 3 - 509	10/23/87

For your information there is an additional monopool, Type 3-503, which has passed all safety testing. However, this material has low potency and will not be considered for release.

Your prompt attention to this matter would be appreciated.

Sincerely,

D. K. McClintock

D. K. McClintock, Ph.D.
Director, Regulatory Affairs
and Quality Control and
Responsible Head
Lederle Biologicals

DKM:pvz
cc: Dr. P. Albrecht
Dr. E. Fitzgerald

October 3, 1962

Dr I. S. Danielson
Lederle Laboratories
Pearl River, New York

Dear Dr. Danielson:

In accord with our telephone conversation I am sending you herewith by Air Mail Special Delivery without refrigeration 5 ml. of the type 3 virus that was used as seed for the large lots prepared for me by Merck, Sharp and Dohme Research Laboratories in 1956. The material I am sending you is designated - Leon $12a_1b$ - KP 3 of 10/10/56.

I should like to point out that this preparation was negative for SV 40 in tests carried out by Dr. Hilleman and his associates, but he told me at the time the tests were made they were not observing the cultures for as long as they are now and he could not be certain that there may not be a trace of SV 40 virus in this material. I should also like to indicate that each ml. of this seed virus should be sufficient for the preparation of 5,000 ml. of seed lot virus.

With best wishes and kindest regards.

Sincerely yours,

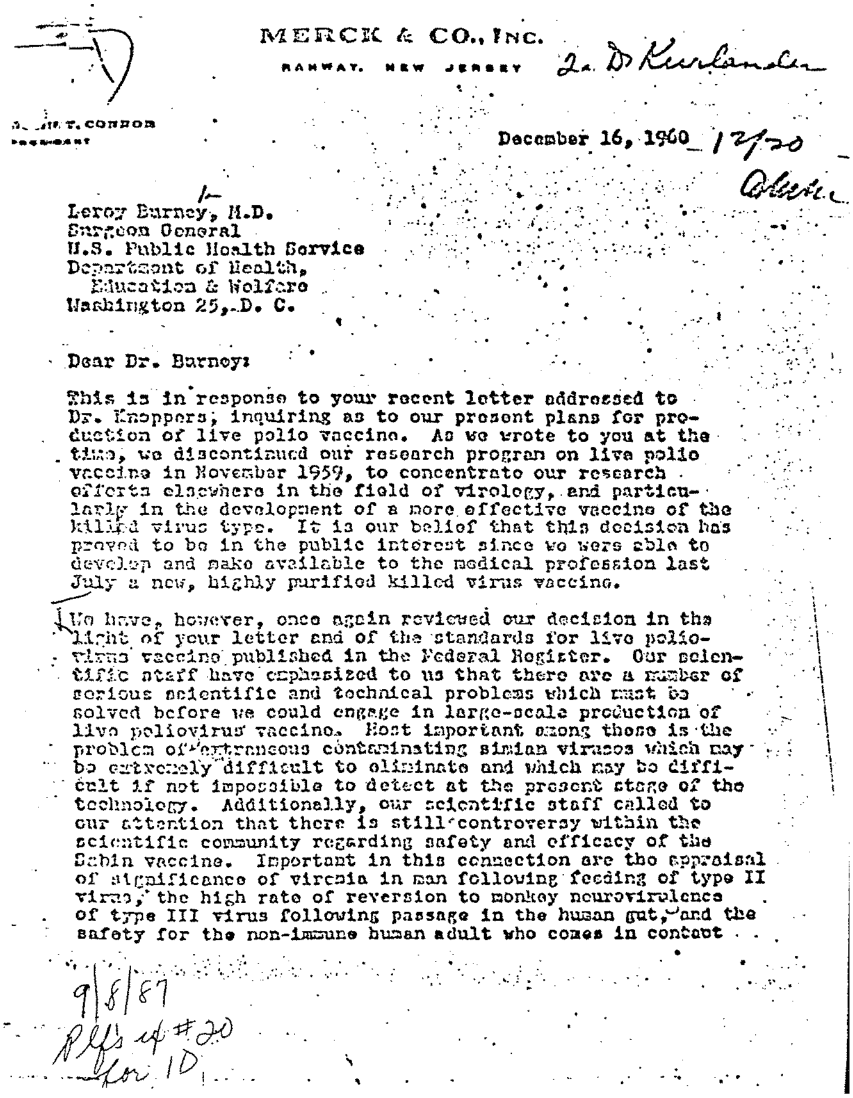
Albert Sabin

Albert B. Sabin, M. D.

ABS:meh

P. S. I would greatly appreciate it if you would acknowledge receipt of this material.

CC: Mr. S. Alston



MERCK & CO., INC.
RAHWAY, NEW JERSEY

[illegible] T. CONNOR

2. Dr Kurlander

December 16, 1960 12/20

Leroy Burney, M.D.
Surgeon General
U.S. Public Health Service
Department of Health,
Education & Welfare
Washington 25, D. C.

Dear Dr. Burney:

This is in response to your recent letter addressed to Dr. Knoppers, inquiring as to our present plans for production of live polio vaccine. As we wrote to you at the time, we discontinued our research program on live polio vaccine in November 1959, to concentrate our research efforts elsewhere in the field of virology, and particularly in the development of a more effective vaccine of the killed virus type. It is our belief that this decision has proved to be in the public interest since we were able to develop and make available to the medical profession last July a new, highly purified killed virus vaccine.

We have, however, once again reviewed our decision in the light of your letter and of the standards for live poliovirus vaccine published in the Federal Register. Our scientific staff have emphasized to us that there are a number of serious scientific and technical problems which must be solved before we could engage in large-scale production of live poliovirus vaccine. Most important among these is the problem of extraneous contaminating simian viruses which may be extremely difficult to eliminate and which may be difficult if not impossible to detect at the present stage of the technology. Additionally, our scientific staff called to our attention that there is still controversy within the scientific community regarding safety and efficacy of the Sabin vaccine. Important in this connection are the appraisal of significance of viremia in man following feeding of type II virus, the high rate of reversion to monkey neurovirulence of type III virus following passage in the human gut, and the safety for the non-immune human adult who comes in contact

9/8/87
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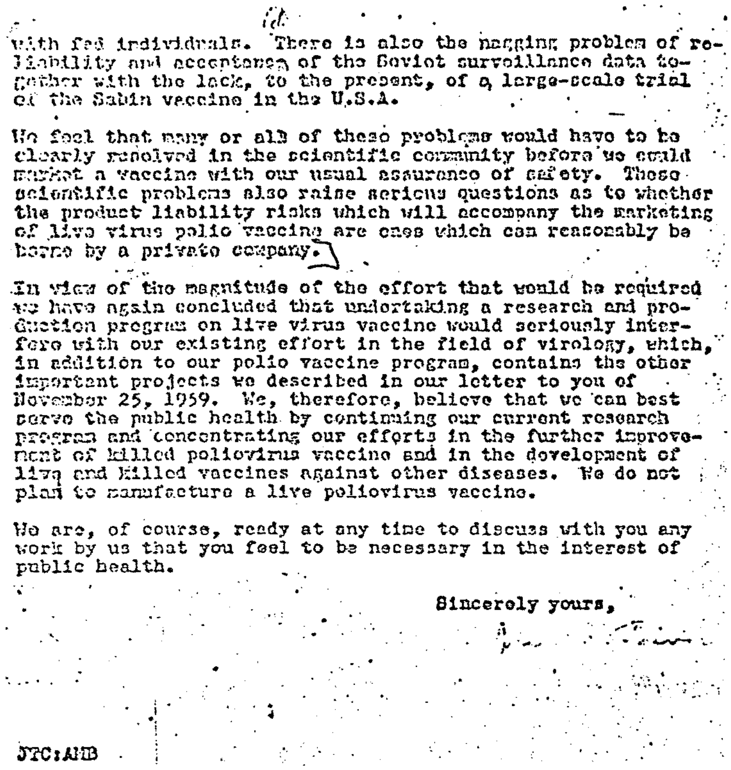
for ID



with fed individuals. There is also the nagging problem of reliability and acceptance of the Soviet surveillance data together with the lack, to the present, of a large-scale trial of the Sabin vaccine in the U.S.A.

We feel that many or all of these problems would have to be clearly resolved in the scientific community before we could market a vaccine with our usual assurance of safety. These scientific problems also raise serious questions as to whether the product liability risks which will accompany the marketing of live virus polio vaccine are ones which can reasonably be borne by a private company.

In view of the magnitude of the effort that would be required we have again concluded that undertaking a research and production program on live virus vaccine would seriously interfere with our existing effort in the field of virology, which, in addition to our polio vaccine program, contains the other important projects we described in our letter to you of November 25, 1959. We, therefore, believe that we can best serve the public health by continuing our current research program and concentrating our efforts in the further improvement of killed poliovirus vaccine and in the development of live and killed vaccines against other diseases. We do not plan to manufacture a live poliovirus vaccine.

We are, of course, ready at any time to discuss with you any work by us that you feel to be necessary in the interest of public health.

Sincerely yours,

JTC:AHB

CYANAMID

Memorandum

To: Mr. W.P. Cekleniak (2)

Location:

From: Mr. S.S. Aiston

Location:

Extension:

Subject: ORIMUNE®, Request for Additional Information for Registration in Australia

Date: March 14, 1979

Reference: Letter from Dr. Dodson, NBSL - BS 79/1 of 9 Feb. 1979
Memo from Dr. Hellestrand to Mr. Cekleniak 16 Feb. 1979

Copy to:
Dr. R.J. Vallancourt
Dr. F.L. Bach*
Dr. C.M. Benitz*
Mrs. D.A. Christian*
Dr. G. Kalish*
Dr. R.J. Saldarini*

3/16/79

CONFIDENTIAL

MUCH OF THE INFORMATION PROVIDED IN RESPONSE TO DR. DODSON'S LETTER SHOULD BE HELD IN STRICTEST CONFIDENCE BY THE AUSTRALIAN BUREAU OF HEALTH.

Copies of Poliovirus Seed charts showing their passage relation to the Sabin Original (SO) seed or Sabin Original/Merck (SOM) as well as all monopools produced from these seeds to date are provided. These seed charts will be referred to several times in this report. (Appendix 1)

As one can see from the type 1 chart, Page 1, Lederle seed 45B-51, which represents two passages from the Sabin Original Merck (SOM), has been in constant use from 1962 to date. The vaccine produced from this seed is the third passage from SOM.

The type 2 seed chart, Page 2, shows that 45B-52, which represents two passages from SOM, has also been in constant use from 1962 to date. The vaccine produced from this seed is the third passage from SOM.

From the study of the type 3 seed chart, Page 3, it can be seen that Lederle had considerable difficulty with this virus, particularly with vaccine that was three passages from SOM from a neurovirulence standpoint.

BERKOVITZ V. LEDERLE PROPRIETARY

Further, many of the monopools that were only two passages from SOM caused difficulty.

In 1963, Lederle obtained a very small amount of the Sabin Original (SO) Seed provided by Dr. Sabin to Merck.

This virus, known as Leon 12 a$_1$b KP3 (10/19/56), was used to produce three Lederle Seed pools 45B-76, 45B-77 and 45B-85. (45B-76 and 45B-77 are no longer in use.) Vaccine produced from these Seed Pools, two passages from (SO), has consistently shown acceptable neurovirulence results.

A copy of a paper by Sabin and Boulger, J. Bio. Std. 1, 115-118, 1973 which describe these seed virus isolation and attenuation is attached. (Appendix 2)

Copies of testing results for extraneous agents and neurovirulence of Lederle seed pools are attached. (Appendix 3)

Copies of methods and results of field trials with Lederle Sabin Trivalent Vaccine are attached. (Appendix 4)

Please refer to the Poliovirus seed charts (Appendix 1) for the relationship between the seed lots and batches of vaccine used in these trials.

It should be made clear that Lederle did not test the original Sabin seeds for extraneous agents or neurovirulence since only 50 ml or less of each seed were provided by Dr. Sabin. It was presumed that if progeny of these seeds proved to be free of extraneous agents and have satisfactory neurovirulence the parent seeds were satisfactory.

Data provided by the Center for Disease Control, U.S. Department of Health, Education and Welfare, Public Health Service, list a possible 162 vaccine associated cases from 1961 through 1977, in the U.S.

This data includes cases following the use of Monovalent and Trivalent Vaccine manufactured by Lederle, Pfizer and Wyeth.

During this same period of time, over 600,000,000 doses of Poliovirus Vaccine, Live, Oral, were distributed by these manufacturers in the U.S. A copy of a revised method of manufacture and testing is attached. (Appendix 5) Also, a copy of the neurovirulence test monograph is attached. (Appendix 5A)

BERKOWITZ v. LEDERLE
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A copy of the most recent Code of Federal Regulations is attached. (Appendix 6)

Copies of the Lederle package insert in use at the present time are attached. (Appendix 7A & 7B)

The Lederle ORIMUNE® package inserts are at present being revised to reflect suggested changes in wording on dosage, adverse reactions and contraindications as published by the Advisory Committee on Immunization Practices, Recommendations of the Public Health Service, Center for Disease Control October 7, 1977 Vol. 26, No. 40. (Appendix 8)

A copy of the Evaluation of poliomyelitis Vaccines by the Institute of Medicine, National Academy of Science is attached for your information. (Appendix 9)

These last two references attest to the safety and efficacy of the Lederle Sabin Vaccine.

The Public Health Service reference poliovirus is tested for intrathalamic and intraspinal neurovirulence every three months as required by the regulations.

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Since 1963 when NA-2 was the reference there have been NA-3 and now NA-4. All three of these references have been tested extensively, before and after acceptance by the Public Health Service, as a reference.

The frequent testing of the reference assures the continuing sensitivity and reproducibility of this most critical test of the vaccine.

The separate attachment, which caused some of the confusion in what neurovirulence testing was done by Lederle, was part of an effort to standardize and clarify interpretation of the intrathalamic test, in 1963. These guidelines have since then, been replaced by further restricted acceptance levels of neurotropisim permitted in both the intrathalamic and intraspinal neurovirulence tests.

Both the intrathalamic and intraspinal tests have always been required by the U.S. Public Health Service, and conducted by Lederle.

Dr. Dodson's question concerning the relative neurovirulence of the original seed viruses and recent monopools is difficult to answer since we have no comparative data.

However, if one considers that the references tested over the years have almost always demonstrated a low level of neurotropic activity -- as they are intended to do -- and recent monovalent pools are satisfactorily passing the neurovirulence tests, one may assume that there has not been a change in relative neurovirulence of the seed viruses since their progeny have low neurovirulence.

Further, one would not expect seeds stored at sub-zero temperatures to change in neurovirulence.

LEDERLE PROPRIETARY

The Code of Federal Regulations permit any monkey of the genus Macaca to be used for the neurovirulence test. Lederle has in the past used only the species Macaca mulatta, or Rhesus, to test the Sabin strains of poliovirus.

With the recent embargo on the exportation of Rhesus monkeys from India and Bangladesh, it is not clear what steps will be necessary to take in the future to solve this problem, although considerable thought and effort is being addressed to the problem.

The origin and history of each monkey are part of the records kept at Lederle, but are not part of the neurovirulence test description. This information could be retrieved for any test if required.

If one will look at the poliovirus seed charts, Appendix 1, page 1, type 1: Lederle did not break consistency until pool 1-217.

Page 2, type 2:
Lederle has not broken consistency with this type.

Page 3, type 3:
Lederle had considerable difficulty maintaining consistency during 1951 and 1962 using seed derived from type 3 SOM.

Produced by
Lederle:
v. Lederle

#3/8

Page 4, type 3:
Since Lederle started using seed derived from the

-4-

(a) The first occurred with pool 3-431. After several attempts to re-establish this seed, it was abandoned because the seed potency was below that required by regulations (21 CFR 630.10(b)(4))

(b) The second occurred with pools 3-450 and 3-451. Consistency has again been re-established.

Questions that Dr. Dodson may have regarding the potency of Lederle Poliovirus Vaccine, Live, Oral, Trivalent, may be answered by reviewing our product description (Appendix 5), the package inserts (Appendices 7A,7B) and 21 CFR 630.17(c) (Appendix 6).

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It is hoped that these responses and the attached information will answer Dr. Dodson's questions.

If further information is requested, please let us know.

Stewart Aiston

Stewart Aiston
Technical Superintendent
Polio Operations

SSA:rb

#318

Produced by
Lederle:
Williams v. Lederle

Pearl River, N. Y. July 3, 1962

Dr. B. W. Carey
Mr. J. C. Blauvelt
Dr. J. M. Ruegsegger

Telephone Call from
Dr. Herman Rosenblum

On July 3, 1962 Dr. Herman Rosenblum, 1400 West 8th, Wilmington, Delaware, (Olympia 4-1627), called. He reported that Dr. Joe Stokes had given a talk to a group of pediatricians on live, oral polio vaccine. He came away very confused. Apparently, Dr. Stokes raised serious doubts in the minds of doctors attending this meeting as to the wisdom of administering oral vaccine.

The doubts were centered around SV_{40} and reversion of Type 3 after human passage. He said that Dr. Stokes has a great influence on the thinking in their area. I reviewed the requirements which deal with the detection of SV_{40}, as well as other simian agents. I agreed that it has been reported that there is some reversion of Type 3 after human passage, as measured by the intracerebral monkey test. I also stated that it is my understanding that the reversion noted was after one passage, while the second and third passages by contact did not seem to progressively increase the reversion.

I suggested to Dr. Rosenblum that he might call Dr. Roderick Murray and ask these same questions since he might be considered a neutral person. I also stated that, if what he learned from Dr. Murray was much different from what I told him, I would appreciate hearing about it.

I told Dr. Rosenblum that I would report our conversation to our clinical group and they may wish to contact him further. Would it be well to organize a meeting in his area? Dr. Stokes has been working with Koprowski.

He stated that we do not say in our package circular that our vaccine is free of SV_{40} while Pfizer's does. I said that I had not even thought of this because the regulations require that demonstrable SV_{40} cannot be present; therefore, it went without saying that our vaccine is SV_{40} free.

ISD:mr

I. S. Danielson

ANTICANCER RESEARCH 20: 4745-4750 (2000)

Oral Polio Vaccine and Human Cancer: A Reassessment of SV40 as a Contaminant Based upon Legal Documents

STANLEY P. KOPS

Abstract. *To date, the scientific literature and research examining SV40 and cancer-related diseases has been based upon an assumption that SV40 was not present in any poliovirus vaccine administered in the United States and was removed from the killed polio vaccine by 1963. The basis for this presumption has been that the regulations for live oral polio vaccine required that SV40 be removed from the seeds and monovalent pools ultimately produced in the manufacturing process. The Division of Biologic Standards permitted an additional two tissue culture passages -- from three to five -- in order to allow manufacturers the ability to remove this contaminant from the oral poliovirus vaccines then awaiting licensure. The confirmation of the removal by one drug manufacturer, Lederle, has been made public at an international symposium in January 1997, where its representatives stated that all of Lederle's seeds had been tested and screened to assure that it was free from SV40 virus. However, in litigation involving the Lederle oral polio vaccine, the manufacturer's internal documents failed to reveal such removal in all of the seeds. The absence of confirmatory testing of the seeds, as well as testimony of a Lederle manager, indicate that this claim of removal of SV40 and the testing for SV40 in all the seeds cannot be fully substantiated. These legal documents and testimony indicate that the scientific community should not be content with prior assumptions that SV40 could not have been in the oral polio vaccine. Only further investigation by outside scientific and independent researchers who can review the test results claimed in the January 1997 meeting and who can conduct their own independent evaluations by testing all the seeds and individual mono-valent pools will assure that SV40 has not been present in commercially sold oral poliovirus vaccine manufactured by Lederle.*

In recent scientific papers in regard to mesothelioma and the role of SV40, the Simian Virus found in polio vaccines, (1-5) the authors restated a premise that has been uniformly accepted in the scientific literature in regard to SV40: that prior to 1963, SV40 was a possible contaminant in poliovirus vaccine, but after 1963, it was no longer a contaminant. Other cancers, brain and bone tumors, have also been associated with SV 40 infection being detected in the tissues. (6) Though these and other articles have stated that both inactivated vaccine and oral polio vaccine were free of SV40 after 1963, that premise warrants re-examination in regard to the oral vaccine manufactured by at least one of the three licensed to produce live oral polio virus vaccine manufacturers.

At the conference held in January of 1997 at which the NIH, the FDA and the CDC met in Bethesda, Maryland joined by scientists from around the world, Dr. M. R. Hilleman of the Merck Institute reviewed the historical record of when SV40 was discovered as a polio vaccine contaminant and what was done to assure its removal differentiating between oral and killed poliovirus products. (7). That meeting was reviewed in Carbone, *et al* 1997a (8).

Dr. Hilleman stated that live poliovirus vaccine manufacturers were required to assure that their product was free of adventitious agents/extraneous microbial agents -- including SV40 -- from the start of the manufacturing process, as this was mandated by the regulations. SV40 had already been detected prior to the licensure of the first oral poliovirus vaccine in the United States and had already been discussed at the Pan American Health Conference, PAHO, held in June of 1960 (9). In August 1960, meetings were held between potential vaccine manufacturers, members of the Surgeon General's committee, and officials of the Division of Biological Standards, part of the National Institutes of Health, which was the regulatory body, all of whose scientists were helping to write the proposed regulations which were to govern oral polio virus vaccine manufacture (10).

In August of 1960, the proposed regulations provided that the oral polio vaccine sold in the United States could not be more than three tissue culture passages beyond the original strain material (11). But when the regulations were enacted in March of 1961, the language of the final regulations had been amended, permitting up to five tissue culture passages (12). The final regulations which became law on March 25, 1961

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Key Words: Polio vaccine, human cancer, SV40.

0250-7005/2000 $2.00+.40

provided that virus in the final vaccine shall represent no more than five tissue culture passages from the original strain, each of which shall have met the criteria of acceptability prescribed in Sec. 73.110(b). Whether SV 40 was removed from Sabin Oral Polio Virus strains, remains a serious and unanswered question.

On March 17. 1961, James Shannon, M.D., then the Director of the National Institutes of Health, advised the Surgeon General of the United States. Luther L. Terry, M.D., that the proposed regulations had been amended and two additional tissue culture passages were being recommended. Permitting five passages was to enable the manufacturers to remove SV40 from the Sabin original strains of the three types of oral live poliovirus vaccine and would permit the seeds to be free of SV40 and any other adventitious agents. "This recommendation was made by the Surgeon General's Committee on Poliomyelitis control based upon its judgment that the removal of adventitious agents particularly the vacuolating and foamy, will necessitate more than three virus passages and that five passages will not have an adverse effect upon the vaccine" (13).

The Federal Regulations issued on March 25, 1961 also required that "Each seed virus used in the manufacture shall be demonstrated to be free of extraneous microbial agents" (12). Similar language can be found in every subsequent modification of these regulations. (14) The term "manufacture" as contained in the Regulations is defined as follows: "Manufacturing" means all steps in propagation or manufacture and preparation of products and includes but is not limited to filling, testing, labeling, packaging, and storage by the manufacturer (15).

In the January 1997 meeting in Bethesda, Maryland, a paper was presented by the manufacturer of the oral polio vaccine, Lederle, which would lead those who participated at the meeting, and the readers of the paper that followed that meeting, to believe that all polio vaccines produced by that company after 1961 were free of the contaminant SV40, or as Lederle put it, "That all subsequent working seed strains have been prepared in CMK cells and screened to assure that they are free from SV40 virus" (16). The Brock paper describes in detail the methods claimed to have been utilized in the testing and screening by Lederle for SV 40. At that meeting for which a transcript does exist, Lederle discussed in detail the procedures for neurtralizing the SV 40, testing for SV 40 and the method utilized (17). This discussion by Lederle does not indicate that any tissue culture passages was utilized to remove SV 40, but rather antisera was utilized in the preparation of the master viral strains. "Master viral strains (seeds) have been prepared in the presence of SV 40 virus antiserum. All subsequent working seed strains have been prepared in CMK tissue and screened to assure they're free of SV 40 virus." There is no indication in this description, or in the Brock paper subsequently published, that modern molecular techniques were utilized in this screening process.

In the course of litigation conducted on behalf of persons with paralysis claimed to have resulted from exposure to the live Orimune polio vaccine product manufactured by Lederle, documents were obtained indicating that some of these statements claiming removal of SV40 from the vaccine seeds should be re-examined and critically reviewed. The content of these legal documents question the assumption that oral polio vaccine produced after 1961 by at least Lederle, one of the three United States manufacturers, was fully tested at every stage of the manufacturing process and that the results of these tests indicate that they were free of SV40.

Between 1961 and 1976 there were three manufacturers of oral poliovirus vaccine in the United States. In the early 1970's, Wyeth Laboratories withdrew its vaccine from the marketplace. By the end of 1976, Pfizer stopped manufacturing vaccine for sale in the United States. From the latter part of 1977 until the end of 1999, only Lederle has manufactured this product for the United States market. In the year 2000, the Centers for Disease Control no longer recommended the use of Oral Polio Vaccine in the United States (18). Whether it is still being manufactured and available for sale, is unknown by this author.

In an internal document of Lederle dated November 8, 1961, approximately eight months after the live oral polio virus regulations became effective, test results disclosed three out of the first fifteen vaccine pools it had utilized to secure both its monovalent license (in 1962) and its trivalent license (in 1963) may have contained SV40 at the PCB 2 level. A monovalent dose of oral polio vaccine contains only one type of each of the three types of polio viruses. A trivalent dose is where all three types of oral polio virus vaccines are combined into a single dose. The specific pools that were identified as having this possible contaminant were lots number 114 of Type I, 216 of Type II and 317 of Type III. All three of these pools were utilized in vaccine commercially sold for several years following licensure in the United States, both in the monovalent and trivalent form of the product (19). Dr. Roderick Murray, Director of the Division of Biologic Standards, the regulatory agency who has the ultimate authority to enforce the federal regulations for all vaccines manufactured and sold in the United States, from 1961 until 1972, was aware, according to this internal documentation, of the presence of SV 40.

The PCB 1 level is attained when the pooled fluids are taken from 25% of the production control vessels at the time of harvest. The PCB 2 level is when pooled fluids are taken from 25% of the production control vessels 14 days after viral inoculation of the production vessels. All of this testing is mandated in the regulations and becomes part of the final protocol submitted to the government for approval.

The Lederle representatives in the January 1997 meeting described the mandated testing as follows: "Viral harvest samples are sent to the quality control laboratory for evaluation and the rest of the harvested fluids are stored frozen until testing is completed. Fluids from these bottles are again tested to detect the presence of any transmissible microbial agent in CMK for 14 days, followed by a subculture in CMK for another 14 days. Viral harvest fluids are also tested again in Rhesus monkey kidney cells, rabbit kidney cells, and BSC-1 cells, all for 14 days. Samples are also tested to demonstrate the absence of microplasma. Quality assurance releases a virus harvest for further processing when all testing has been completed with satisfactory results - for the original cell culture, the cell culture fluid testing and subcultures, and the viral harvest samples. In summary, over four thousand individual cell culture observations are made during the quality control testing of a single trivalent bulk lot. Any product contamination observed at any point, results in rejection" (17).

The licensing lots utilized by American Cyanamid for both its monovalent and trivalent licenses were Type I, lots numbered 113 to 117; for its Type II, lots numbered 213 to 217; and for its Type III, lots numbered 313 to 317 (20).

Testimony of Dr. Mary Ritchey in 1998, then Vice President of Operations for the Wyeth-Lederle Vaccines and Pediatric Business Group, was that American Cyanamid could not now determine that all of the polio vaccine seeds and strains were tested for SV40, as Lederle did not have protocols in its possession for all of its strain and seed materials (21). Dr. Ritchey testified that there were no protocols for any of the three master seeds, Type I, Type II and Type III. (21) She testified there were no protocols for any of the following seed numbers: 3101, 3102, 3107, 1102, 45B51, 2107, and 45B52 (21). Dr. Ritchey also testified that over the years, American Cyanamid had utilized intermediate seeds, in addition to the aforementioned seeds, in the manufacturing process of its oral polio virus vaccine. There are no records that these seeds were tested for SV40 (21). It is this history which brings into question the assumptions heretofore made by the scientific community based upon prior assurances of safety testing and results that indicated that all of the seeds had passed the screening process as stated in the January 1997 meeting in Bethesda, Maryland.

In documents submitted to Congress prior to the licensing of the Orimune product and prior to the enactment of the regulatory system governing the production and sale of the oral polio vaccine, Merck & Co. specifically declined to manufacture the Sabin vaccine, in part because of its concerns about the question of the contaminant SV40. In a letter addressed to the Surgeon General of the United States, Merck & Co. stated the following:

"We have, however, once again reviewed our decision in the light of your letter and of the standards for live poliovirus vaccine published in the Federal Register. Our scientific staff have emphasized to us that there are a number of serious scientific and technical problems that must be solved before we could engage in large-scale production of live poliovirus vaccine. Most important among these is the problem of extraneous contaminating simian viruses that may be extremely difficult to eliminate and which may be difficult if not impossible to detect at the present stage of the technology" (22).

Dr. Albert Sabin had been advised that the testing performed by Merck & Co. on his original seed strains for the presence of SV40 may not detect the presence or absence of SV40, and so informed American Cyanamid in correspondence in 1962 (23).

In 1979, American Cyanamid's technical superintendent of polio vaccine production, when preparing to submit documents to a foreign licensing authority, stated the following in an internal memoranda: "It should be made clear that Lederle did not test the original Sabin seeds for extraneous agents or neurovirulence since only 50 ml or less of each seed were provided by Dr. Sabin" (24).

The import of this testimony and the referenced Lederle internal documents challenge the conclusions previously advanced in scientific journals that people born after 1963 who tested positive for SV40 either became contaminated as a result of human-to-human transmission, or of placental transference. The scientific community, having been assured by the principal, and eventually sole, OPV manufacturer that SV40 had been removed never conducted research to determine whether or not that was accurate. No investigation is contained anywhere in the literature to verify the results of testing of all of the seeds of the Orimune manufacturer, Lederle, by independent scientific investigators to determine whether the seeds, including intermediate seeds, were free of SV40.

There has been no scientific literature which has reviewed and critically analyzed the Lederle documents submitted to the government and/or Lederle's own internal documents showing that each and every seed, including intermediate seeds, were tested and free from SV40 contamination.

There has been no scientific investigation to determine whether or not the post-1963 mesothelioma and other cancerous condition cases in which SV40 has been detected or isolated from tissue samples were caused by vaccine that individual was given or through contact with someone who had been recently administered a vaccine that may have contained the SV40 contaminant.

The scientific community should now require an inquiry into whether all the seeds utilized in production of the Orimune product were tested for SV40 and what the test results revealed for each of the seeds utilized in the manufacturing process, including intermediate seeds. Unfortunately, there is no documented evidence that either Lederle or the FDA could produce to substantiate the claim that all the seeds were tested and that they all passed the mandated standards. In litigation, demands were made both on the United States in the *In Re Sabin Litigation*, and on American Cyanamid in numerous cases now pending, and the documents still have not been furnished, showing the testing of each and every seed utilized by American Cyanamid in its manufacturing process for the product Orimune. Further investigation should be conducted to determine whether any of the seeds of each of the three types of the monovalent pools comprising the trivalent vaccine given to every newborn in the United States for decades did have SV40 as a contaminant in any of the strains, seeds and monovalent pools. Only if all tests are negative can there be any assurance that SV40-contaminated vaccine did not reach the vaccinees and/or their contacts.

The scientific literature indicates that mesothelioma can be initiated, promoted, and/or accelerated by a combination of various factors including SV40. This breakthrough in scientific research now requires a complete appraisal of whether the oral vaccine used in the United States from 1961 until 2000 could have been a contributing cause. The determination of where the SV40 which has been isolated in post-1961 cases in conjunction with various cancers came from is an essential step in continuing any meaningful scientific investigation into these cancers.

The answer to many of the questions raised herein is available from the vaccine manufacturer or the regulatory agency, presently the Food and Drug Administration. They can furnish their records and the seed stocks and monovalent pool stocks in their storage facilities to the scientific community. They should furnish to the scientific community their internal records including all test results showing that each of the strain, seeds and intermediate seeds, as well as the monovalent pools derived therefrom, without exception, were tested and that these results revealed that SV40 had been successfully and completely eliminated from oral polio vaccine.

Federal reg-u-la-tions required that protocols be kept of all tests performed by the manufacturer, including those to determine whether or not SV40 was present in its product. (25) In an affidavit filed in another litigation case, Lederle swore that every seed was submitted to the government for the government's review and approved by the government. (26) This sworn statement, and the Brock paper (16) and presentation (17) conflicts with the sworn testimony of Dr. Ritchey (21). Only demands by the scientific community requesting the actual documents, protocols and test results can fully answer this apparent contradiction. If these protocols exist, then the data should be made available for review to the medical community at large. If they do not exist, then the medical community should be so informed.

Capability to test with more precision and accuracy for SV40 has increased as scientific knowledge has advanced. (27) Therefore, testing of seed material, including intermediate seed material and monovalent pools, should be performed to determine whether today's enhanced testing technology reveals the presence of SV40 in any product and in any seed that was used in the manufacturing process from 1961 until the present.

Full details of how the SV40 present in the oral polio vaccine was removed from the original strains, seeds and final product, should now be fully disclosed to the scientific community. Only then can epidemiologic and oncologic analysis and discussion of the role, if any, of SV40 in tumor processes be based upon a fully informed scientific community which is the best available information on this important area of on-going scientific study and research.

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11 Proposed Regulations, August 1960. Plaintiff's Exhibit No. 43 - In Re Sabin Polio Vaccine Litigation, MDL 780, U.S.D.C., MD – Baltimore, Maryland.

12 Federal Register, Saturday, March 25, 1961 at page 2565-2568, Sec. 73.110, et seq.

13 Memorandum from Director of N.I.H. to Surgeon General of the United States dated March 16, 1961. Defendant's Exhibit No. 33 - In Re Sabin Polio Vaccine Litigation, MDL 780, U.S.D.C., MD – Baltimore, Maryland.

14 21 Code of Federal Regulations 630.10(b)(3): "Each seed virus used in manufacture shall be demonstrated to be free of extraneous microbial agents except for unavoidable bacteriophage."

15 21 Code of Federal Regulations, 600.3(u).

16 Brock, Kelleher and Zlotnick, Developments in Biologic Standardization, Simian Virus 40 (SV40): A Possible Human Polyvirus; Product Quality Control testing for the Oral Polio Vaccine. 217-219, 1998.

17 Transcript of Proceedings 300-307 of the Developments in Biological Standards, January 1997.

18 Recommendation of the CDC's Advisory Committee on Immunization Practices, June 17, 1999.

19 Lederle internal memorandum from Dr. Biddle to Dr. Danielson dated November 8, 1961, submitted in response to discovery requests in the polio litigation.

20 Letter of submission of American Cyanamid to the United States dated November 6, 1961. Defendant's Exhibit No. 57- In Re Sabin Polio Vaccine Litigation, MDL 780, U.S.D.C., MD – Baltimore, Maryland.

21 Testimony of Mary Ritchey, Ph.D., Vice President of Operations, Wyeth-Lederle Vaccines and Pediatrics Business Group, dated April 14, 1998, in the U.S.D.C. for the E.D. of Ohio, Civil Action No. 94-423 and 94-425.

22 Letter from John T. Conner of Merck & Co. to Dr. Leroy Burney, Surgeon General of the United States, dated 12/16/60 – Plaintiff's Exhibit No. 54 - In Re Sabin Polio Vaccine Litigation, MDL 780, U.S.D.C., MD – Baltimore, Maryland.

23 Letter from Albert Sabin, M.D. to Dr. I.S. Danielson, Lederle Laboratories, dated October 8, 1962 – Plaintiff's Exhibit No. 178 - In Re Sabin Polio Vaccine Litigation, MDL 780, U.S.D.C., MD – Baltimore, Maryland.

24 Lederle internal memorandum dated March 14, 1979 – Plaintiff's Exhibit No. 483 - In Re Sabin Polio Vaccine Litigation, MDL 780, U.S.D.C., MD – Baltimore, Maryland.

25 21 Code of Federal Regulations § 630.17(e).

26 Sworn affidavit in Stuart v. American Cyanamid Co., U.S.D.C., S.D.N.Y. 95 Civ. 4940 dated 2/5/96 by Lederle's Senior Director and Responsible Head Regulatory Affairs and Technical Assessment.

27 Rizzo P, DiResta I, Powers A, Rattner H, and Carbone M. Unique strains of SV 40 in commercial poliovaccines from 1955 not readily identifiable with current testing for SV 40 infection. Cancer Res. *59:* 60103-6108, 1999.

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Re: Debate on the Link Between SV40 and Human Cancer Continues

The news article by Nancy J. Nelson *(1)* repeats the current scientific dogma that simian virus 40 (SV40) was removed from all oral polio vaccine sold and administered in the United States. In a recent article *(2)*, however, I have challenged this accepted "fact" based on legal documents and the absence of test results from at least one of the principal vaccine manufacturers, Lederle. As noted in that article, internal Lederle documents indicate that the company has not been able to document that it tested all vaccine seeds to confirm the absence of SV40 contamination. Therefore, statements in Nelson's article, such as "[p]eople most likely to have received contaminated vaccines were born from 1941 through 1961," are inaccurate and potentially misleading.

Dr. Strickler's statement that "[m]esotheliomas are developing in people who are too old to be vaccinated, and brain tumors [are developing] in children that are too young to have been vaccinated," may be explained by the presence of SV40 in the oral polio vaccine and the fact that oral polio vaccine can spread from the recipient to those who come in contact with the excretions (oral and fecal) of the recipient within a defined period of time *(3)*. There has been no investigation of whether SV40 can be transmitted from individuals vaccinated with the live oral polio vaccine to unvaccinated individuals because everyone has assumed that SV40 was never in that product from the inception of its being sold in the United States.

Every scientist who is attempting to determine the role of SV40 as a cause of cancer in humans and every news reporter who is interested in this issue should demand all of the records of both the government and the vaccine manufacturer so that there can be a full scientific and independent investigation as to whether there was full compliance with the removal of SV40 from all oral polio vaccine used in the United States from 1962 until 2000. Oral polio vaccine is no longer *sold* in the United States, and only enhanced inactivated vaccine is now allowed for routine immunization.

STANLEY P. KOPS

REFERENCES

(1) Nelson NJ. Debate on the link between SV40 and human cancer continues. J Natl Cancer Inst 2001;93:1284–6.

(2) Kops SP. Oral polio vaccine and human cancer: a reassessment of SV40 as a contaminant based upon legal documents. Anticancer Res 2000;20:4745–9.

(3) Henderson DA, Witte JJ, Morris L, Langmuir AD. Paralytic disease associated with oral polio vaccines. JAMA 1964;190:41–8.

NOTES

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Overall, Nancy Nelson's news article about simian virus 40 (SV40) and human tumors *(1)* was well balanced. We would like to make some additional comments. (More information can be found in the February 2001 issue of *Seminars in Cancer Biology*.)

First, the statement in the sidebar that SV40 causes "abnormalities" in human cells underestimates the extent of damage. Human cells infected by SV40 *in vitro* develop extensive genetic damage [*(2)*, *reviewed in (3)*] and have grown as tumor nodules when injected into volunteers *(4)*. The susceptibility of human cells to SV40 is cell type dependent, with mesothelial cells the most susceptible *(2,3,5)*. SV40 is the only known carcinogen that, by itself, causes malignant transformation and immortalization of human mesothelial cells in tissue culture *(2,3,5)*.

Second, the incidence of mesothelioma in the United States has increased from nearly none in 1955 to approximately 2500 cases per year, and SV40 may be one of the contributing factors.

Third, the hypothesis that most mesotheliomas occurred in an age group that could not have been exposed to SV40-contaminated polio vaccines *(1)* reflects a common but mistaken belief that, during the "contamination" period (1955 through 1961), only newborns and children were vaccinated. In the United States, 34.7 million people aged 20–59 years were vaccinated with potentially contaminated polio vaccine during this period *(3)*. This is the cohort in which most mesotheliomas have developed in the past 20 years *(3)*. Furthermore, administration of the oral-attenuated polio vaccine in the early 1960s exposed both the recipient and his contacts to the poliovirus and to SV40 because both viruses were infectious. There is also evidence that SV40-contaminated vaccines were produced and distributed after 1961 *(6)*. Finally, even if polio vaccines contributed to the spread of SV40, other mechanisms of transmission presumably exist because SV40 has been detected in nonvaccinated individuals *(3)*.

Fourth, epidemiology studies [reviewed in *(3)*] have measured the association of the polio vaccine with the overall increase in cancer. However, one would not expect increases in rare cancers such as mesotheliomas to affect overall cancer rates. Two independent studies of cohorts vaccinated in early childhood with potentially contaminated polio vaccines *(3)* suggested an increased risk of mesothelioma (relative risk, ≥3). However, the number of cases was small because mesotheliomas are rare in people younger than 50 years; thus, it is premature to make definitive conclusions about an increased risk of mesothelioma in individuals vaccinated with SV40-contaminated polio vaccines *(3)*.

Fifth, several groups have used various technical approaches (immunostaining, messenger RNA *in situ* hybridization, and primed *in situ* hybridization) to demonstrate that SV40 is present in malignant mesothelioma cells but not in nearby normal cells *(3)*. Moreover, SV40 has been detected in mesothelioma cells and not in nearby nonmalignant cells microdissected from the same slide *(3)*.

Sixth, SV40 is not always lost when mesothelioma cells are cultured *(5)*; accordingly, treatment with an antisense construct to the SV40 T antigen arrests SV40-positive mesothelioma cells in tissue culture [reviewed in *(7)*]. Also, Epstein-Barr, another episomal virus, is often lost when human nasopharyngeal tumor cells are put in tissue culture.

Seventh, it has recently been shown that a small number of mesothelioma

Immunization Safety Review:
SV40 Contamination of Polio Vaccine and Cancer

Immunization to protect children and adults from many infectious diseases is one of the greatest achievements of public health. Immunization is not without risks, however. It is well established, for example, that some influenza vaccines have been associated with a risk of Guillain-Barré syndrome and that vaccines sometimes produce anaphylactic shock. Given the widespread use of vaccines, state mandates requiring vaccination of children for entry into school, college, or day care, and the importance of ensuring that trust in immunization programs is justified, it is essential that safety concerns receive assiduous attention.

The Immunization Safety Review Committee was established by the Institute of Medicine (IOM) to evaluate the evidence on possible causal associations between immunizations and certain adverse outcomes, and to then present conclusions and recommendations. The committee's mandate also includes assessing the broader significance for society of these immunization safety issues.

In this fifth report in a series, the committee examines the hypothesis that exposure to polio vaccine contaminated with simian virus 40 (SV40) can cause certain types of cancer.

THE CHARGE TO THE COMMITTEE

Challenges to the safety of immunizations are prominent in public and scientific debate. Given these persistent and growing concerns about immunization safety, the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) recognized the need for an independent, expert group to address immunization safety in a timely and objective manner. The IOM has been involved in such issues since the 1970s. (A brief chronology can be found in Appendix C.) In 1999, as a result of IOM's previous work and its access to independent scientific experts, CDC and NIH began a year of discussions with IOM to develop the Immunization Safety Review project, which addressed both emerging and existing vaccine safety issues.

The Immunization Safety Review Committee is responsible for examining a broad variety of immunization safety concerns. Committee members have expertise in pediatrics, neurology, immunology, internal medicine, infectious diseases, genetics, epidemiology, biostatistics, risk perception and communication, decision analysis, public health, nursing, and ethics. While all the committee members share the view that immunization is generally beneficial, none of them has a vested interest in the specific immunization safety issues that come before the group. Additional discussion of the committee composition can be found in the Foreword written by Dr. Harvey Fineberg, President of the IOM.

The committee is charged with examining three immunization safety hypotheses each year during the three-year study period (2001–2003). These hypotheses are selected by the Interagency Vaccine Group, whose members represent several units of the Department of Health and Human Services (DHHS)—the National Vaccine Program Office, the National Immunization Program, and the National Center for Infectious Diseases at the CDC, the National Institute for Allergy and Infectious Diseases at the NIH, the Food and Drug Administration (FDA), the Na-

American Academy of Pediatrics, and the American Academy of Family Physicians all now recommend exclusive use of IPV to immunize children and adults in the United States (CDC, 2000). The committee found no indication of any concern that SV40 contamination of IPV in the United S tates in use today might have escaped detection. Therefore, **the committee does not recomm end a policy review of polio vaccine by any of the national or federal vaccine advisory bodies on the basis of concerns about cancer risks that might be associated with exposure to SV40, because the vaccine in current use is free of SV40.**

Claims have been made that some oral polio vaccines might have been contaminated after 1963 (Kops, 2000). The committee urges that FDA or other agencies address these claims to try to resolve the uncertainty regarding the possibility of exposure to SV40 after 1963. Appropriate assumptions about exposure are essential for conducting valid epidemiologic analyses of the risks that might be associated with contaminated OPV.

Policy Analysis and Communication

The ability of researchers to use epidemiologic studies to assess the possible association of SV40 with certain cancers has been hindered in part by the small numbers of cases for most of the cancers of concern, but also by substantial uncertainty in classifying individuals as exposed or unexposed.

The uncertainty as to exposure arises in large part because of lack of detailed information on the levels of SV40 in the vaccine and on who received the vaccine. Testing of stored samples of IPV used in 1955 showed that the levels of SV40 varied among vaccine lots and that some lots were not contaminated (Fraumeni et al., 1963). But samples of vaccine produced through the remainder of the period of likely contamination (1955-1961) were not available for testing, leaving investigators with little basis for identifying differences in SV40 exposure among the vaccinated population. Misclassification of exposure would result in a bias toward the null hypothesis of no difference between the "exposed" and "unexposed" groups.

The committee hopes that contamination of a vaccine never occurs again, but also considers it prudent to have a comprehensive plan in place for prevention of contamination, as well as for response and communication should such an event occur. Pieces of such a plan already exist within the various agencies with responsibility for assuring the safety of vaccines. For example, FDA has regulatory authority over the production of vaccines. Currently, all vaccines licensed by the FDA are required to fulfill general safety, sterility, and purity requirements (Code of Federal Regulations, 2001). For example, the cell substrates used to produced the MMR vaccine are from flocks free of the avian leukosis virus. The MMR vaccine also undergoes testing for adventitious viral activity. Cell lines used to produce the poliovirus vaccine are tested for (in addition to SV40) tuberculosis, herpes viruses, measles, and other infectious agents (CDC, 1997). However, the committee is not aware of a comprehensive system that is transparent and clearly understandable to, and accessible by, the public. The most recent comprehensive plan put together by the federal government on vaccine safety does not address contamination issues (NIH, 1998).

The committee recommends that the appropriate federal agencies develop a Vaccine Contamination Prevention and Response Plan. The appropriate agencies should be given the authority and resources to implement the plan once it is in place. This plan should identify the procedures already in place or those that need to be developed, for example, to prevent contamination of vaccine during the manufacturing process. In addition, the plan should include strategies for routine assessment of vaccine for possible contamination; notification of public health officials, health care providers, and the public if contamination occurs; identification of recipi-

SV40 contamination of vaccines

- Oral polio-vaccines were required to be free of SV40 since 1961
- Injected polio-vaccines were required to be free of SV40 since 1961, but contaminated vaccines were sold until 1963
- During litigation against Lederle I obtained documents indicating that SV40 was present in their product well after 1961

Documents obtained during litigation

- These documents indicate that the Lederle product was never freed from SV40
- That Lederle did not follow the mandatory Code of Federal Regulations
- That Lederle knowingly distributed SV40-contaminated vaccines
- That Lederle never tested whether the neutralization procedure worked

Documents from litigation

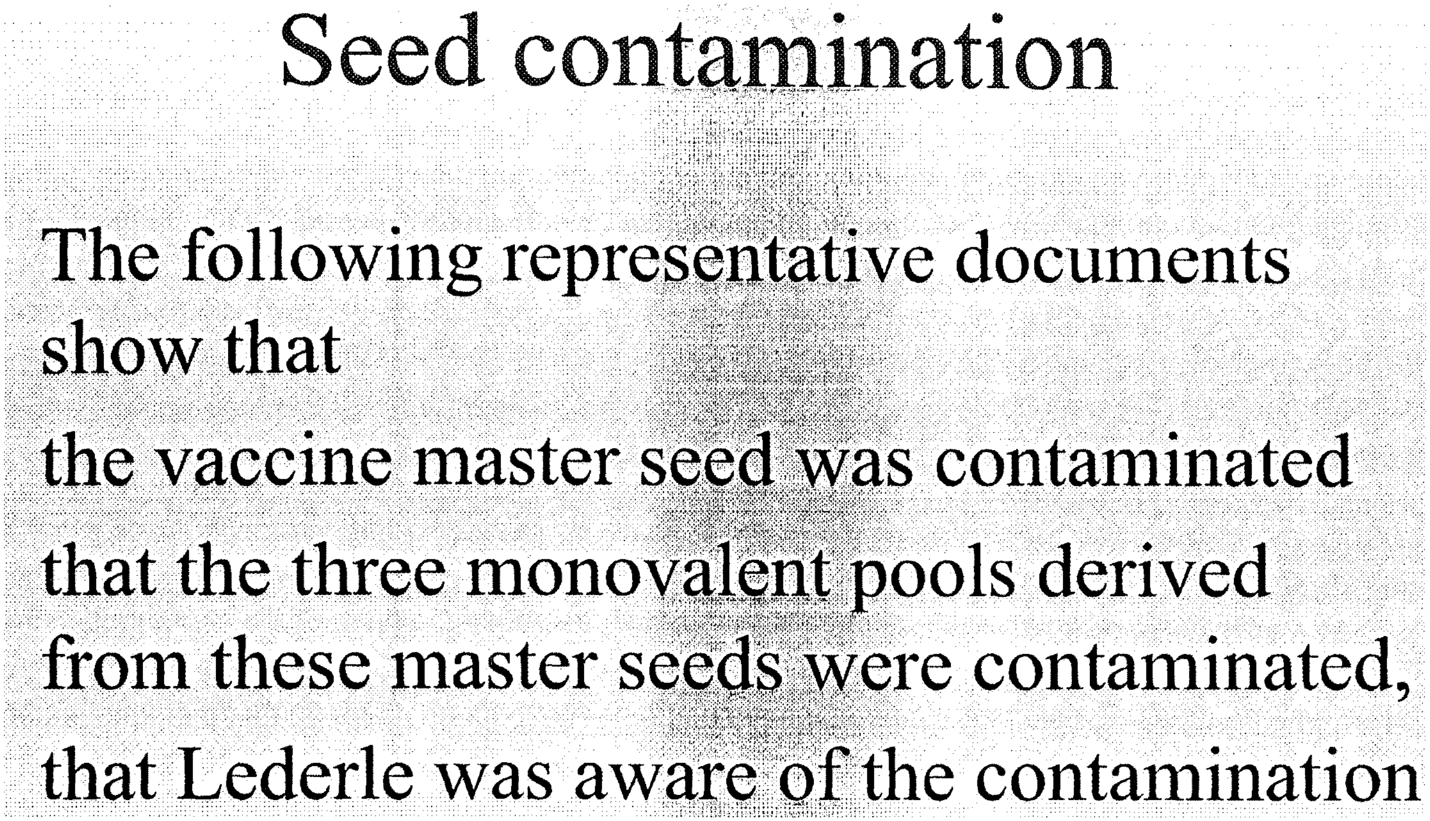
- Lederle put me under a confidential order which prohibits me to distribute most of these documents
- Therefore, if you want to see the Lederle documents proving what I will state, you need to ask them directly or to ask them to allow me to show the documents to you

Sources of SV40 contamination

- Vaccines can be contaminated because the seed used to manufacture is contaminated
- Because the monkey kidney cells used to manufacture are contaminated

How contamination is detected

- Contamination is detected by observing the appearance of vacuoles or other cytopathic effects in cells incubated with tissue culture media and/or seed



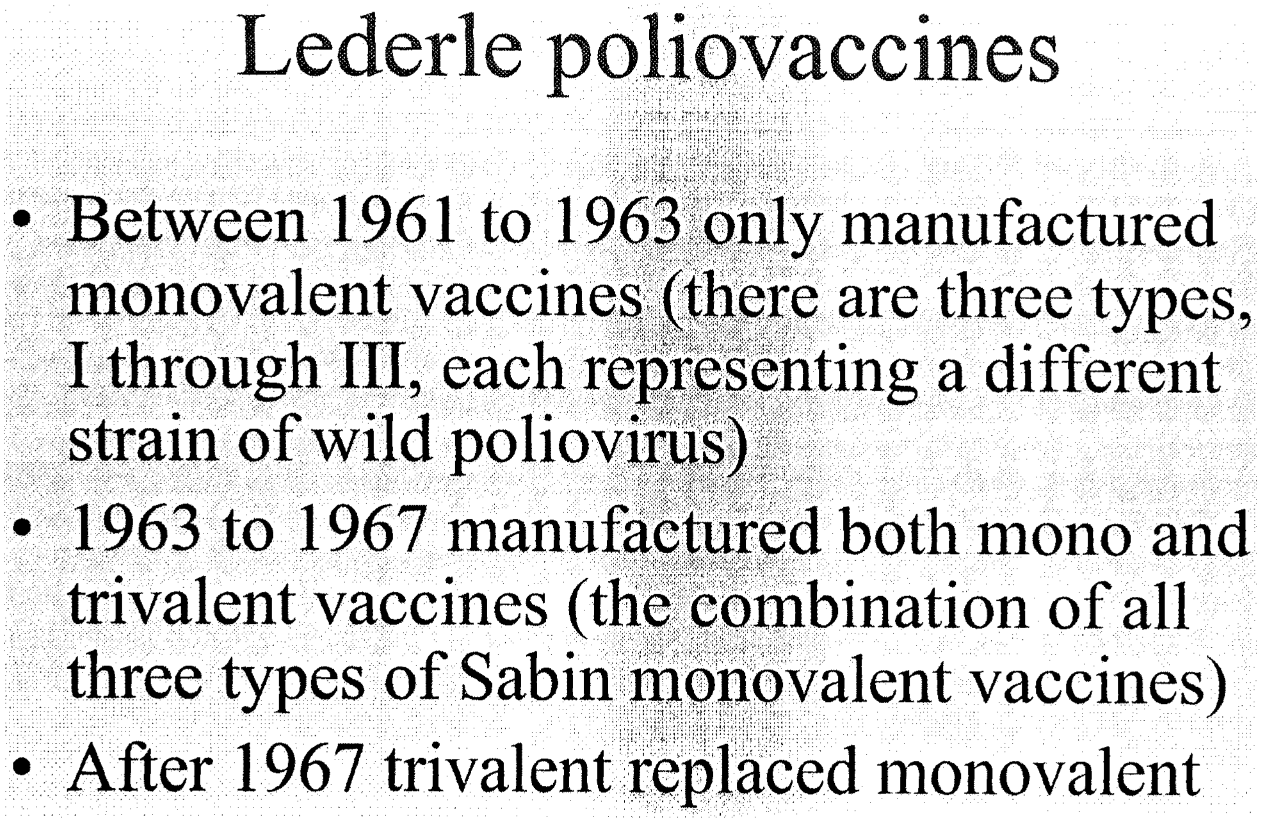
Seed contamination

The following representative documents show that

the vaccine master seed was contaminated

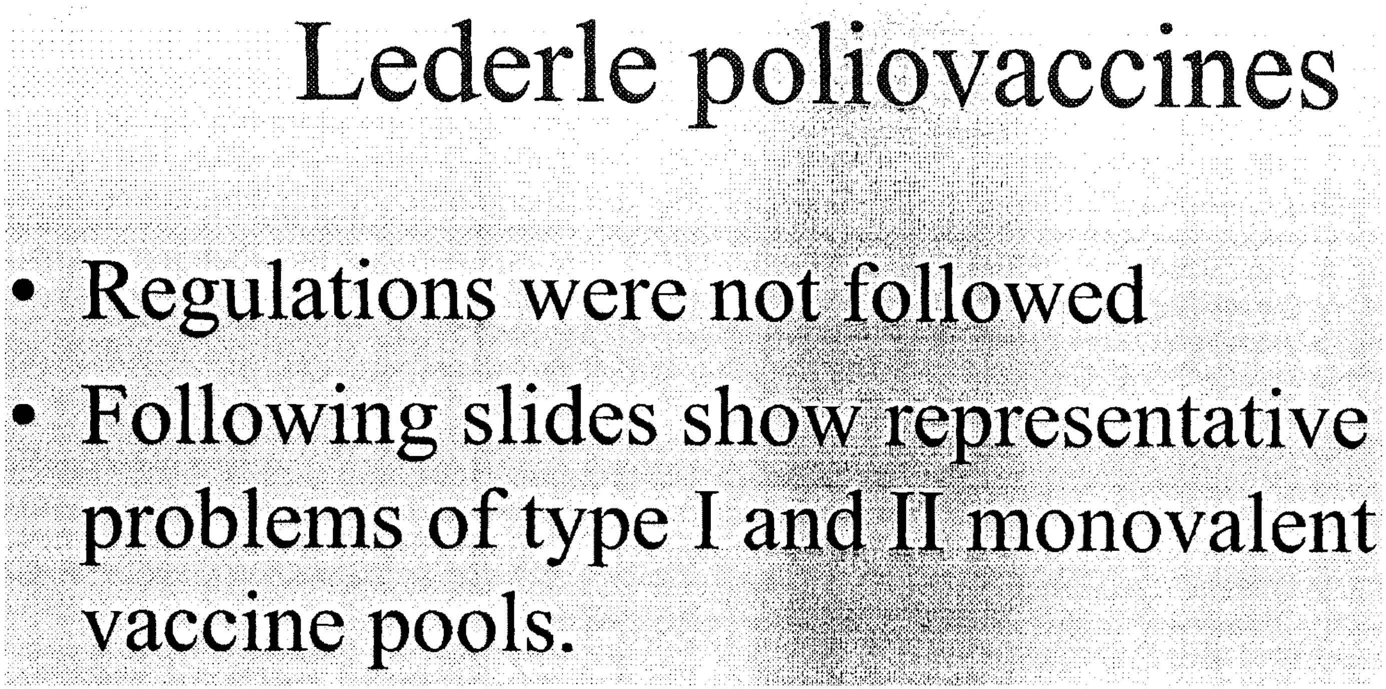
that the three monovalent pools derived from these master seeds were contaminated,

that Lederle was aware of the contamination



Lederle poliovaccines

- Between 1961 to 1963 only manufactured monovalent vaccines (there are three types, I through III, each representing a different strain of wild poliovirus)
- 1963 to 1967 manufactured both mono and trivalent vaccines (the combination of all three types of Sabin monovalent vaccines)
- After 1967 trivalent replaced monovalent

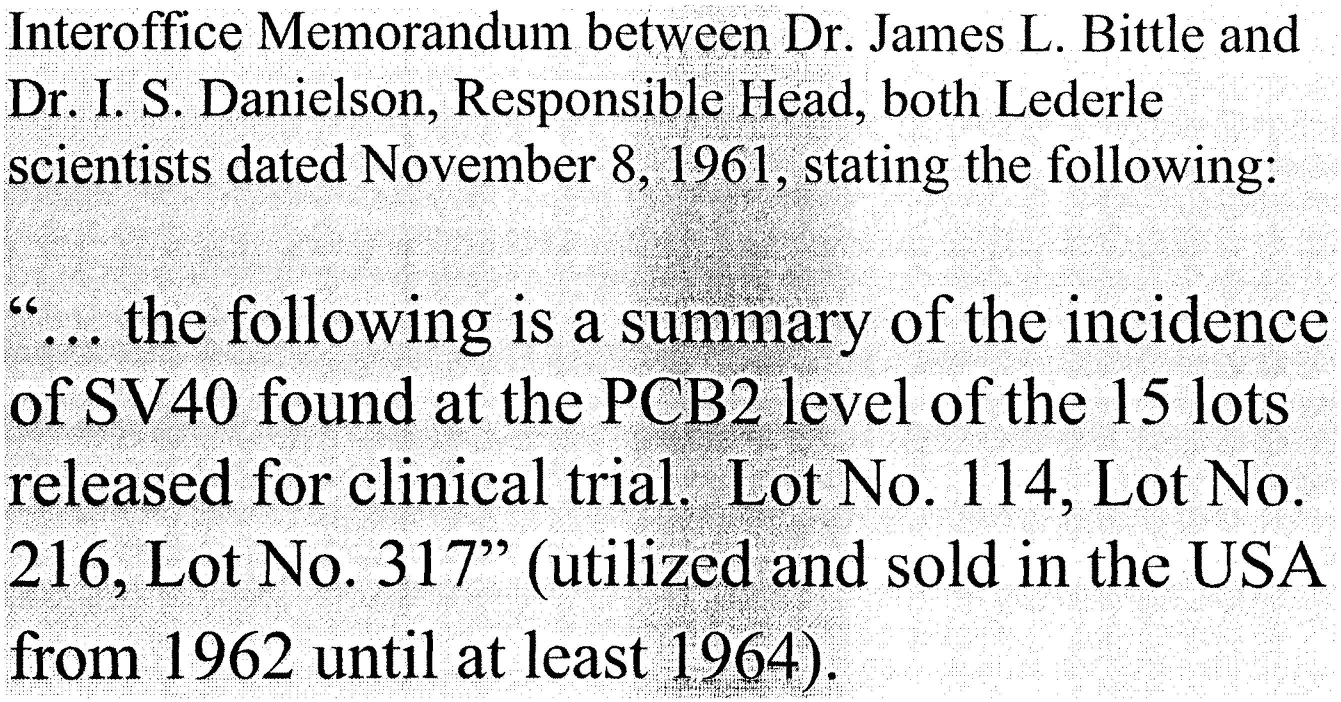


Lederle poliovaccines

- Regulations were not followed
- Following slides show representative problems of type I and II monovalent vaccine pools.

Interoffice Memorandum between Mr. S. S. Aiston, Technical Superintendent of Polio Production Lederle and Mr. W. P. Ceklenіak, dated March 14, 1979, states the following:

“It should be made clear that Lederle did not test the original Sabin seeds for extraneous agents or neurovirulence since only 50 ml or less of each seed were provided by Dr. Sabin. It was presumed that if progeny of these seeds proved to be free of extraneous agents and have satisfactory neurovirulence the parent seeds were satisfactory”.



Interoffice Memorandum between Dr. James L. Bittle and Dr. I. S. Danielson, Responsible Head, both Lederle scientists dated November 8, 1961, stating the following:

"… the following is a summary of the incidence of SV40 found at the PCB2 level of the 15 lots released for clinical trial. Lot No. 114, Lot No. 216, Lot No. 317" (utilized and sold in the USA from 1962 until at least 1964).

MANUFACTURING RECORD – TYPE I – MONOPOOL
114 – HARVEST NO. 1110 – RHESUS MONKEY J071 – PCB 2

Pooled Fluids taken from 25% Production Control Vessels
Fourteen Days after Viral Inoculation of Production Vessels
Sec. 73.113 Code of Federal Regulations. **(Slide 1 of 3)**

	CPE Observed on Day			
	Sample		TC Control	
	6	14	6	14
CMK 1.17.61	0	CPE	0	0
(Retest)	0	CPE	0	0
CMK 3.21.61	0	0	0	0
(Retest)	0	0	0	0

MANUFACTURING RECORD – TYPE I – MONOPOOL 114

(Slide 2 of 3)

Subculture of test made 1.17.61

Test System	Sample		TC Control #CMK-59		TC Control #CMK-30	
CMK 30	4	14	4	14	4	14
	VA		0		0	
	VA		0		0	
	VA		0		0	
	VA		0		0	
	VA		0		0	
	VA		0		0	
	VA		0		0	
	VA		0		0	

MANUFACTURING RECORD – TYPE I – MONOPOOL 114

(Slide 3 of 3)

Subculture of retest made 3.21.61

Test System	Sample	TC Control #CMK-27	TC Control #CMK-26
CMK 26	(5)	(5)	(5)
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0

MANUFACTURING RECORD – TYPE II – MONOPOOL 216 – HARVEST NO. 2128 – RHESUS MONKEY L087 – PCB 2

Pooled Fluids taken from 25% Production Control Vessels Fourteen Days after Viral Inoculation of Production Vessels Sec. 73.113 Code of Federal Regulations.**(Slide 1 of 2)**

	CPE OBSERVED ON DAY			
System	Sample		TC Control	
CMK 118	10	14	10	14
	0	VA	0	0
	0	VA	0	0
CMK 122	0	VA	0	0
	0	VA	0	0

MANUFACTURING RECORD [illegible] TYPE II – MONOPOOL [illegible]216

Subculture of pooled fluids of CMK-122 tested 6.7.61

(Slide 2 of 2)

Test System	Sample	TC Control #CMK-26	TC Control #CMK-122
CMK 80	4	4	4
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0
	VA	0	0

Monkey contamination

- To prevent contamination manufacturers stated that all working seeds were prepared in SV40-free green monkeys
- Their own test showed that 10% of those monkeys were infected with SV40
- Furthermore, Rhesus monkeys were used to prepare type I and II seeds from 1961-1980.

Conclusions 1

- Seeds were prepared in rhesus kidney tissue and not African green monkey tissue for type I and II. This increases the risk of contamination
- 10% of green monkeys were SV40 infected
- Seeds were not tested for SV40
- Some seeds were not neutralized for SV40
- Seeds neutralized were not tested to see if the neutralization worked

Conclusions 2

- SV40 contamination was detected in all 3 monovalent types by Lederle
- Lederle ignored contamination and proceeded to release contaminated vaccines
- This failure to follow regulations continued until 1999